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MySci: A New Approach to Teaching Biological Science Laboratories

Korli Alford

MySci: A NEW APPROACH TO TEACHING BIOLOGICAL SCIENCE LABORATORIES

By

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ABSTRACT

According to the National Science Foundation's report "Vision and Change in Undergraduate Biology Education: A Call to Action," project-based learning creates effective graduates and future collaborators. Instructors who are able to adapt their course to meet the unique interests of their students create graduates who are more likely to engage with peers and to retain the information taught throughout the class. The goal of this project was to develop a course based on student-driven, evidence-based learning. Five major and 12 minor, student-selectable labs were implemented in the initial test reported herein. A total of seven undergraduate students and three graduate students attempted combinations of these labs. Our goal was to reinforce the pervasive nature of the Central Dogma, Transmission Genetics, and the Hardy-Weinberg assumptions in biological sciences research. We report on the success of each lab and discuss the work required to improve on this concept.

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CHAPTER 1

INTRODUCTION

There were three factors that significantly contributed to the development of this project. First, a currently operational course in PCR Methods had been developed that students complete in near autonomy [DeCaire et al., 2015]. Secondly, the "Vision and Change in Undergraduate Biology Education: A Call to Action" [Ledbetter, 2012] indicates the importance of project-based learning in improving outcomes for undergraduates. Third, the availability of instructor-customizable course materials [American College of Healthcare Executives, 2019; Evolve, 2019; W.W. Norton & Company, 2019] led to the basic question "can an advanced course in genetics be taught with student-selectable projects that can be completed in near autonomy?" This project describes our first attempt at this ambitious goal.

Most science teaching laboratories use standardized, step by step procedures which are followed by students at the same time and place. This approach appears to help the lab instructor, but is didactic in nature and reduces independent thought by the student. It is also somewhat dependent on student attendance, requiring the instructor to re-teach the same material multiple times due to (predictable) student absence.

We sought to yield as much independence to each student as possible so that they can learn and apply the scientific method. Because this course is designed to teach genetics, we sought to include applications of the Central Dogma, Transmission

Genetics and Population Genetics. Our goal was to develop an independently executed series of labs (Table 1.1) that promote both independent problem solving and personal responsibility. A collection of five required labs and 12 optional labs was created. The optional labs were selected by students based on student interests (Table 1.1). These optional labs were independently executed by students; undergraduate students were required to complete two and graduate students were required to complete three of these labs.

Table 1.1. Core and Optional Lab Manual Components and Exercises

Lab Title	Lab Components	Lab Exercise
Prokaryote Sequencing	Prokaryote collection, sequencing, and BLAST analysis	"Human Oral Microbiome" worksheet and presentation
Human Population Collection and Description	Collection, PCR, and electrophoresis of human DNA; CODIS fingerprinting of human DNA	"Human Population Collection and Description" worksheet
Plant Population Collection and Description	Collection, PCR, and electrophoresis of plant DNA; Location mapping of plant collection sites; Sequencing and BLAST analysis of plant samples	"Plant Population Collection and Description" worksheet and presentation
Human Gene Investigation	Selection of gene of interest, Primer design; PCR and electrophoresis of human DNA using designed primers, Sequencing of samples	"Human Gene Investigation" presentation; incorporated into final student presentation
Forensics	PCR, electrophoresis, sequencing, and BLAST of plant DNA; CODIS fingerprinting of human DNA	"Forensics" presentation; incorporated into final student presentation
Optional, Student Selectable Labs	Allele Migration, Allele Variation, Gene Structure, Genetic Counseling, Natural Selection, Paternity Testing, Biochemistry, Medical Diagnosis, Microbiology, Parasitology, Pathology, Psychology	

1.1 The Scientific Method

The scientific method is a list of steps used to investigate and/or solve a problem. The first step of the scientific method is identifying a problem to be solved and investigating what is already known (or not known) about this problem. The next step is to identify or create a possible explanation/solution for this problem; this step is often referred to as creating one or more hypotheses. Next, a controlled experiment must be developed in order to test the validity of the hypotheses. After this, the controlled experiment must be conducted, with the investigator collecting data throughout the process. Once data collection is complete, the data must be analyzed in order to determine whether it supports the hypotheses. Lastly, hypotheses are re-formed and re-tested as needed in order to pursue a more thorough solution to the original problem. Scientists are constantly designing experiments, interpreting results, and then developing additional hypothesis-driven questions. This process ultimately leads to the scientific method being utilized again and again throughout the entirety of a scientist's research career.

All STEM fields rely on the scientific method as the standard for producing realistic, reproducible solutions to problems. Since the ultimate goal of STEM experiments is to solve problems, the scientific method needs to be thoroughly understood by all STEM students.

1.2 Transmission Genetics

Transmission genetics describes how genes are inherited or *transmitted* from one generation to the next. Humans have been manipulating transmission genetics since the

beginning of civilization, first by selecting, then by breeding animals and crops. Transmission genetics became a powerful tool when Gregor Mendel's experiments [Mendel, 1866] were re-discovered in the early 20th century [Correns, 1900; de Vries, 1901-03]. Mendel introduced the idea of dominant and recessive factors (alleles) which resulted in the creation of three "principles of heredity," which are used today to describe transmission genetics. The Principle of Uniformity states that all heterozygotes share a common phenotype and that hybrids are uniform in appearance. The Principle of Segregation states that during meiosis each gamete receives and carries one allele from each gene. The Principle of Independent Assortment states that genes separate independently during meiosis, meaning that genes are inherited independently from one another. Although transmission genetics is used to describe the basic concept of heredity, not all inheritance follows Mendelian principles.

1.3 The Central Dogma

The Central Dogma (Figure 1.1) explains the flow of genetic information from DNA to RNA to functional molecules (proteins) and the inheritance of genetic material from generation to generation [Crick, 1958]. DNA replication occurs via mitosis and meiosis. During mitosis, the cell produces new copies of DNA identical to a cell's original copy of DNA. Meiosis includes a process called "crossing over," in preparation for gamete fertilization. Crossing over occurs when homologous chromosomes exchange segments (of the same size) with one another. Ultimately, meiosis results in the creation of unique combinations of DNA molecules for the progeny of the individual.

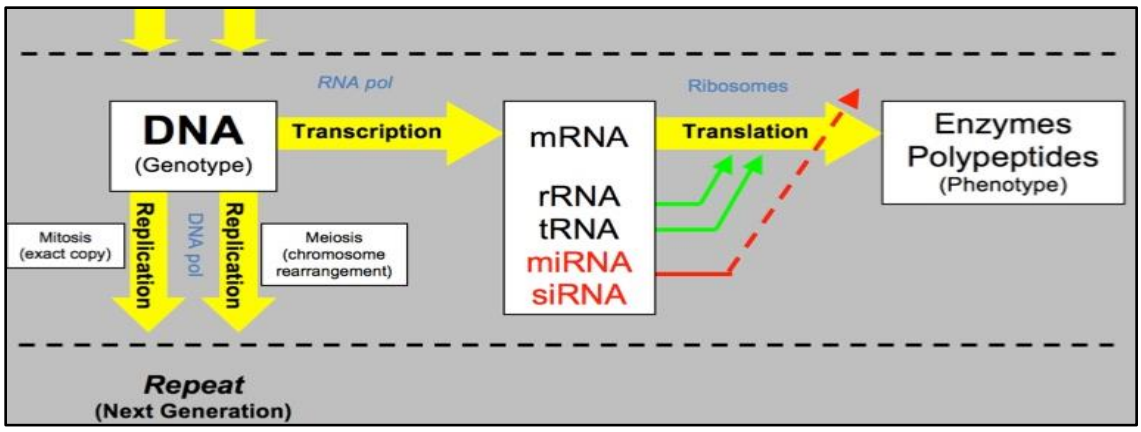

Figure 1.1. A diagram of the Central Dogma. *Provided by Dr. Shultz.*

In active cells, DNA is transcribed into RNA by RNA polymerase and results in the formation of various types of RNA molecules, the most recognized of which are rRNA, mRNA, and tRNA. These RNA molecules work together and ultimately result in RNA being translated into functional amino-acid polypeptides. Transcription can also result in the formation of miRNA and siRNA, both of which regulate the process of translation.

By understanding the major concepts found in the Central Dogma (replication, transcription, and translation), students can better understand why and how an individual's genotype and phenotype are created. Many aspects of genetic diversity can be linked back to an unexpected occurrence during some phase of the central dogma. Therefore, understanding the central dogma makes it easier to analyze and understand almost all other concepts in genetics.

1.4 Population Genetics

Most populations change over time. A population that does not change *must* follow the five Hardy-Weinberg assumptions: no mutation, no natural selection, no migration, random mating, and no genetic drift [Hardy, 1908; Weinberg, 1908]. The genetic variation of a population is the result of combinations of mutations being inherited through multiple generations. Natural selection describes when specific individuals within a population have been selected for or against, with the end result measured in reproductive fitness. Migration indicates that individuals have either moved into or out of a population, which results in differences between populations in separate geographic locations. Non-random mating occurs when a trait is selected for or when related individuals in a population reproduce, and results in an increased frequency of homozygous recessive traits. Genetic drift describes a small population that loses genetic diversity by random chance, founding effects, or bottlenecks. The (rare) population that is not changing is said to be in Hardy-Weinberg equilibrium. Understanding the five Hardy Weinberg assumptions can help a student conceptualize why certain populations have evolved and provides a starting point for creating hypotheses.

1.5 Independent Thinking

All STEM students should be encouraged to think independently and critically in order to become effective members of the scientific community. Often, college courses assess how much a student has learned using standardized, memorization-based testing procedures; they do not assess whether or not students are effective problem-solvers. Inquiry-based learning techniques encourage students to think for themselves, not just

rely on memorized facts, helping students to understand core concepts and to solve problems on their own.

1.6 Group Projects

College courses often have group-based projects with multiple students treated as one entity. It often falls on the most responsible individual of the group to create and execute the entire project. These scenarios are not fair to anyone involved; they promote the idea that as long as an individual has a driven and intelligent leader, the individual can put in as little effort as possible and have no major consequences. Projects based on independent work are therefore preferred.

1.7 Goals for this Project

This project had multiple goals. The first task was to assess the initial development of laboratory exercises that can be independently selected and performed. Second, each completed lab protocol needed to be evaluated to assess how effective it was at teaching students the core concepts of genetics which include: the Central Dogma, Transmission Genetics, and Population Genetics. The third task was to analyze whether each of the protocols are robust enough to stand up to students with a basic skill set and if so, to assess how each protocol improves this basic skill set. Finally, a method of assessing student success and knowledge gained had to be established. The development of educational materials without a final, objective assessment of their expected effect is not ultimately useful. We sought to incorporate these goals into a laboratory course in genetics.

A total of three different hypotheses were tested within this study.

H1, An academic course consisting of independently selectable and executable lab projects unique to each student can be created.

H2, The designed protocols will be effective at incorporating the Central Dogma, Transmission Genetics, and the Hardy-Weinberg assumptions.

H3, The assessment of student skill sets and gain in skills can be accomplished without pre-created answers.

CHAPTER 2

MATERIALS AND METHODS

2.1 Manual Assembly

A total of 17 lab modules were tested throughout this course. Students were required to complete five modules including prokaryote sequencing, human population DNA collection and description, plant population DNA collection and description, human gene investigation, and forensics. Once the required protocols were completed, students completed two (three for graduate students) more modules from a list of 12 options. These prototype labs were in the rough development stage and included: allele migration, allele variation, gene structure, genetic counseling, natural selection, paternity testing, biochemistry, medical diagnosis, microbiology, parasitology, psychology, and pathology. The following sections describe the protocols for each of the major wet labs.

2.2 Prokaryote Sequencing

One each of 100 mm LB and TSA plates were labeled with each student's initials and the date. A disposable, sterile inoculating loop was used to streak each plate. Plates were incubated at 37ºC for 24-72 hours, then photographed. A total of six colonies were selected for sequencing based on colony morphology; morphology of all colonies was cataloged using a colony morphology chart (Figure 2.1). A permanent marker was used to circle and number each selected colony on the outside of the respective plates. The plates were photographed again to show colony growth and selected colonies.

LB Colonies

Colony	Form	Surface	Edge	Elevation
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
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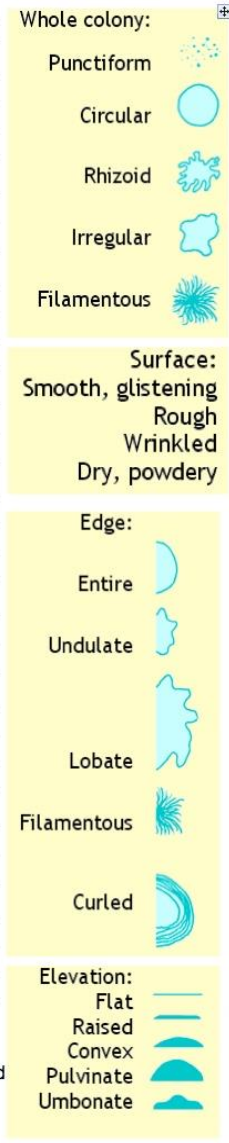

downloaded and modified from: https://commons.wikimedia.org/wiki/ File:Bacterial_colony_morphology.png By No machine-readable author provided. Ewen assumed (based on copyright claims) [Public domain], via Wikimedia Commons

TSA Colonies

Colony	Form	Surface	Edge	Elevation
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
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Figure 2.1. Example of the colony morphology chart completed by each student for project 1.

Colony PCR was performed on the selected colonies. Six 0.2 mL PCR tubes were labeled with student initials and colony numbers. Each reaction contained 2 µL ITS primer, (forward: **TGTAAAACGACGGCCAGT**CCTACGGGAGGCAGCAG; reverse: AATACCGCGGCTGCTGG), 10 µL GoTaq, and 8 µL molecular biology water. The forward primer used in this protocol contained an M13 tag (Figure 2.2), which is

indicated by bold text. Separate pipette tips were used to gently scrape each selected colony and mix the cells collected from each colony into the appropriate PCR tube. Colony DNA was amplified using a BioRad T100 Thermal Cycler, with an initial denaturing step for 5 minutes at 95°, 35 cycles of denaturing for 60 seconds at 95°, annealing for 75 seconds at 50°, and extension for 45 seconds at 72°, and a final extending period for 5 minutes at 72°. All samples were held at 10ºC until removed from the thermal cycler.

After PCR was run, 8µL of each sample was loaded into separate lanes of an agarose gel; 8µL of a molecular marker (proprietary, 100, 250, 500, 750, and 1000bp sizes) was loaded into the first and last wells of each lane on the gel. The gel was run for approximately one hour to determine if the samples produced a ~180bp band. Each lane on the gel was photographed using a UVP documentation system (BioDoc-It Imaging System, M-26). If the sample produced a ~180bp band, the remaining 12µL of the sample was placed into a sequencing tube (MWG Operon, SimpleSeq Reactions); this step was conducted individually for each sample. Student names, colony numbers, and sequencing tube numbers were documented in an Excel spreadsheet. The Basic Local Alignment Search Tool (BLAST), from the National Center for Biotechnology Information (NCBI), was used for 16S ribosomal RNA analysis of each sample's sequencing results [NCBI, 2019]. The e-value (generated by BLAST) of each sample's results was also recorded; the "e-value" indicates the probability of a random match, with 0.0 being the strongest statistical match. Upon completion of the lab 1 protocol, the "Project One: Human Oral Microbiome" worksheet was completed individually by each student; each student also completed an oral presentation of their results for this lab.

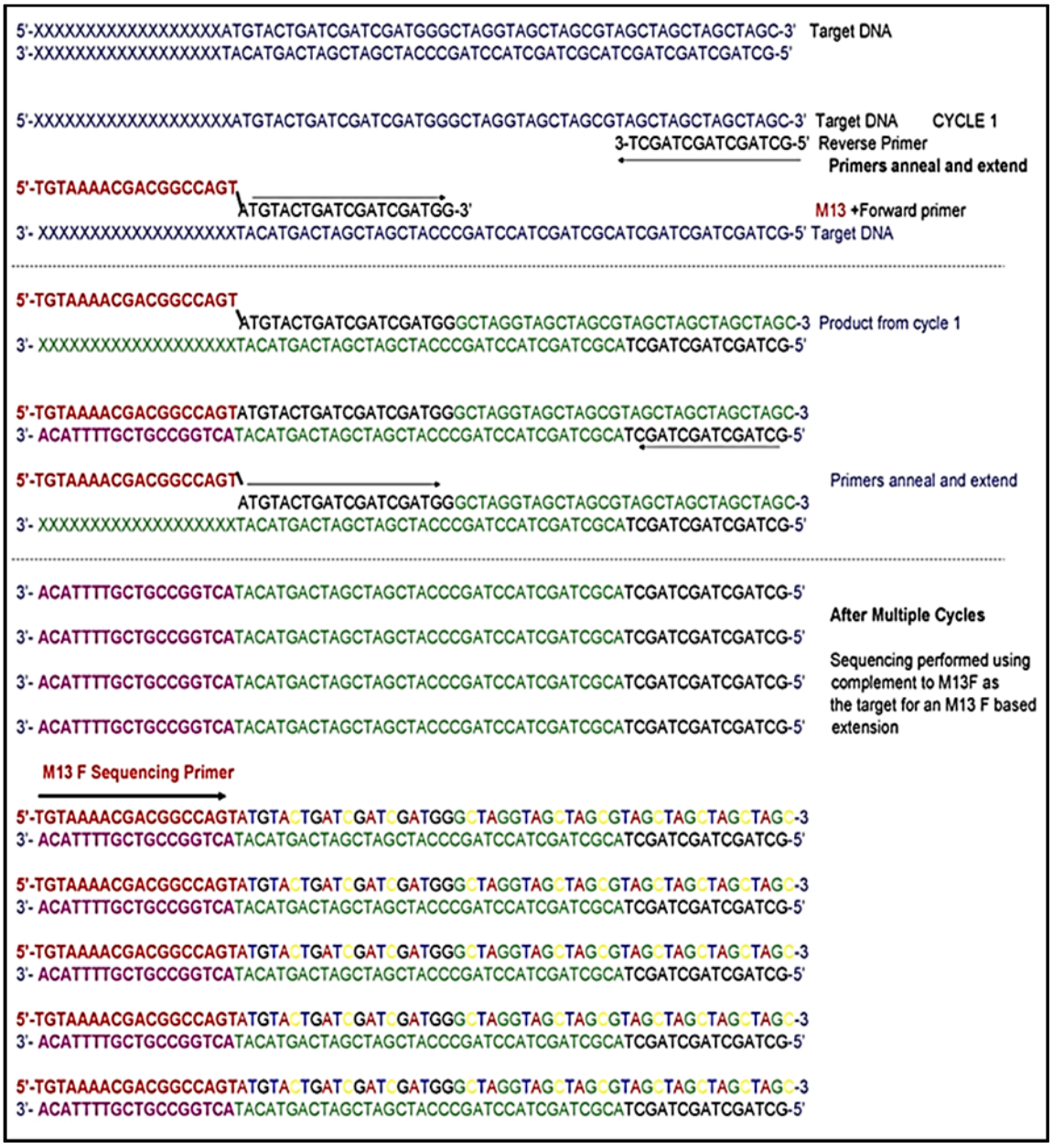


Figure 2.2. Overview of M13 labeled sequencing [DeCaire et al., 2015].

2.3 Human Population Collection and Description

2.3.1 Human DNA Extraction and Collection

Approximately 30mL of a 0.9% saline solution was poured into individual, 3 oz. disposable Dixie cups and given to students. Students rinsed their mouths with the solution for approximately 30 seconds, then expectorated the contents back into their cup.

One-thousand microliters of the solution was pipetted out of the cup and into a 1.5mL tube labeled with student initials. The 1.5mL tubes were centrifuged at maximum speed (14,400 rpm) for 2 minutes by the instructor in a tabletop centrifuge (Beckman Coulter, cat. # A46474). During this process, a pellet of cells forms at the bottom of each tube. If a pellet did not form, the saline was pipetted out of the tube and back into the cup, and another 1000uL was placed into the 1.5mL tube and re-centrifuged. This process was repeated until a pellet was visible.

The supernatant was removed from the 1.5mL tube and pipetted back into the cup. Next, 100uL of Instagene Matrix (Bio-Rad, cat. # 732-6030) was transferred into the 1.5mL tube. Cheek cells were re-suspended in the Instagene Matrix via pipetting. The resulting mixture was then transferred to a 1.5mL screw-cap tube and finger-vortexed. The screw-cap tubes were incubated at 56ºC for 5 minutes, removed from the heat block, finger-vortexed, and then incubated at 56ºC for an additional five minutes. The tubes were then removed from the heat block and shaken several times, then placed into a 100ºC heat block for 5 minutes. After the three incubation steps, the tubes were centrifuged in the tabletop centrifuge at 6000 x g for 5 minutes. Next, the top 100uL of supernatant was pipetted into a blue 2.0mL tube that was labeled with student initials and "stock DNA." A total of 70uL of the mixture in the blue 2.0mL tube was transferred into a red 2.0mL tube; this unlabeled tube was placed in a designated rack in the front of the room. The unlabeled tubes were randomized and transferred to different, randomly labeled tubes at the end of the lab to maintain confidentiality.

2.3.2 Human Fingerprinting

Students were given one sample of human DNA each from the class DNA collection. Master mixes containing 70uL of GoTaq, 23uL of molecular biology grade water, and 36uL of randomized DNA were made in labeled 1.5mL tubes. Seven 0.2mL PCR tubes were labeled with student initials and FGA, TPOX, D7S820, D8S1179, TH01, D21S11, and AMEL.

Next, 17uL of the master mix was pipetted into each labeled PCR tube. A total of 2uL of the appropriate primer (FGA, TPOX, D7S820, D8S1179, TH01, D21S11, and AMEL) was added to each of the 7 PCR tubes (Table 2.1). The PCR tubes were briefly centrifuged and then placed into a BioRad T100 Thermal Cycler using an initial denaturing step for 5 minutes at 95°, 35 cycles of denaturing for 60 seconds at 95°, annealing for 75 seconds at 55°, and extending for 45 seconds at 72°, and a final extending period for 5 minutes at 72°. All samples were held at 10ºC until removed from the thermal cycler.

After the PCR program was run, all of the product was loaded into an agarose gel. After the gel was run, the size of the product for each primer was estimated, then entered (along with the suspect number) into the "CODIS database." A portion of the associated project/ worksheet titled "Project 2: Population Collection and Description" was completed individually by each student upon the completion of this section of the lab 2 protocol.

Table 2.1. CODIS Primer Sequences [Budowle et al., 2001; Eng, Ainsworth, and Waye, 1994; National Institute Standards and Technology, 2019]

CODIS Primer	Product Range (bp)	Primer Sequences
AMEL	X- 977	5'-CTGATGGTTGGCCTCAAGCCTGTG-3'
	Y- 788	5'-TAAAGAGATTCATTAACTTGACTG-3'
CSF1PO	287-331	5'-AACCTGAGTCTGCCAAGGACTAGC-3'
		5'-TTCCACACACCACTGGCCATCTTC-3'
D3S1358	99-147	5'-ACTGCAGTCCAATCTGGGT-3'
		5'-ATGAAATCAACAGAGGCTTG-3'
D5S818	129-177	5'-GGGTGATTTTCCTCTTTGGT-3'
		5'-TGATTCCAATCATAGCCACA-3'
D7S820	194-234	5'-TGTCATAGTTTAGAACGAACTAACG-3'
		5'-CTGAGGTATCAAAAACTCAGAGG-3'
D8S1179	157-209	5'-TTTTTGTATTTCATGTGTACATTCG-3'
		5'-CGTAGCTATAATTAGTTCATTTTCA-3'
D13S317	unlisted	5'-ACAGAAGTCTGGGATGTGGA-3'
		5'-GCCCAAAAAGACAGACAGAA-3'
D16S539	129-177	5'-GATCCCAAGCTCTTCCTCTT-3'
		5'-ACGTTTGTGTGTGCATCTGT-3'
D18S51	262-349	5'-CAAACCCGACTACCAGCAAC-3'
		5'-GAGCCATGTTCATGCCACTG-3'
D21S11	154-272	5'-GTGAGTCAATTCCCCAAG-3'
		5'-GTTGTATTAGTCAATGTTCTCC-3'
FGA	158-314	5'-GCCCCATAGGTTTTGAACTCA-3'
		5'-TGATTTGTCTGTAATTGCCAGC-3'
TH01	171-215	5'-GTGGGCTGAAAAGCTCCCGATTAT-3'
		5'-ATTCAAAGGGTATCTGGGCTCTGG-3'
TPOX	216-264	5'-ACTGGCACAGAACAGGCACTTAGG-3'
		5'-GGAGGAACTGGGAACCACACAGGT-3'
VWA	122-182	5'-CCCTAGTGGATAAGAATAATC-3'
		5'-GGACAGATGATAAATACATAGGATGGATGG-3'

2.4 Plant Population Collection and Description

2.4.1 Plant Sample Collection and DNA Extraction

Students were told that the area for plant DNA collection was the quad on Louisiana Tech University's main campus (Figure 2.3). Students were instructed to decide amongst themselves how to divide the workload and to collect at least one DNA

sample from each plant in the designated area (decorative flowers, grass, and trees were excluded) using the described method. First, students drew a map of the designated plant DNA collection area (all plant locations are noted on the map). Flashcards with plant numbers/labels were created; students were instructed to photograph each of their plants while incorporating the flashcard into the picture. A leaf tissue disk was obtained from each plant using a leaf punch. The disks were placed in labeled 1.5mL tubes, placed on ice and taken back to the lab.

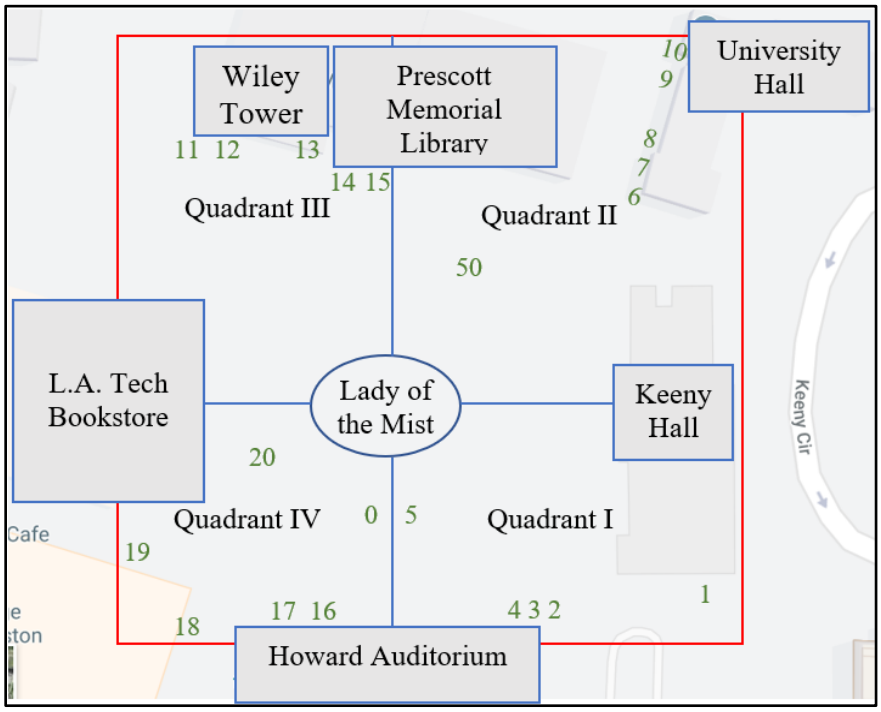


Figure 2.3. Louisiana Tech quad area map used for plant database.

Next, 100uL of extraction solution (Sigma, E-7426) was pipetted into each DNA collection tube. The tubes were centrifuged and then examined to ensure that the leaf punch was completely submerged in the extraction solution. The tubes were placed in a 95ºC heat block and incubated for 10 minutes. The tubes were removed from the heat

block and 100uL of dilution solution (Sigma, D-5688) was pipetted into each DNA collection tube. New 1.5mL tubes were labeled with student initials, plant sample numbers, and "d" (to signify *diluted* DNA). Next, 45uL of molecular biology grade water was pipetted into each dilution tube. Then, 5uL of DNA from the leaf sample tubes were pipetted into separate dilution tubes.

2.4.2 Plant Sequencing

Students were instructed to work independently of one another for this procedure. Each student ascertained the appropriate number of PCR tubes needed to run their plant samples and labeled them with plant sample numbers. A 1.5mL tube was labeled with student initials and used to create a master mix consisting of 3uL of plant-ITS primer (Table 2.2), 15uL of GoTaq, and 9uL of molecular biology grade water (per plant sample). Then, 25uL of the master mix was transferred into each labeled PCR tube.

Table 2.2. Plant-ITS Primer Sequences; M13F Sequence is Indicated by Bold Text

Primer	Primer Direction	Primer Sequence
P3	Forward	5'-**TGTAAAACGACGGCCAGT**YGACTCTCGGCAACGGATA-3'
U1	Forward	5'-**TGTAAAACGACGGCCAGT**GGAAGKARAAGTCGTAACAAG-3'
U4	Reverse	5'-RGTTTCTTTTCCTCCGCTTA-3'

Next, 5uL of each diluted plant sample was transferred into the appropriate PCR tube. The PCR tubes were briefly centrifuged, then placed into a BioRad T100 Thermal Cycler using an initial denaturing step for 5 minutes at 95°, 35 cycles of denaturing for 60 seconds at 95°, annealing for 75 seconds at 55°, and extending for 45 seconds 72°, and a final extending period for 5 minutes at 72°. All samples were held at 10ºC until removed from the thermal cycler.

After the PCR program was run, 10uL of each product was loaded into separate wells on an agarose gel, alongside a ladder. The gel was run for an hour to see if they produced ~500bp or ~750 bp bands. A picture of the gel was taken using a UVP documentation system (BioDoc-It Imaging System, M-26). If a sample was successful, the remaining product from the PCR tube was loaded into a sequencing tube. A list of student initials, plant sample numbers, and sequencing tube numbers was composed on an Excel spreadsheet. A list of plant identification numbers and sequences was also created (Appendix A, Table A1). Students performed a BLAST search on each sample to determine the identity of each plant. A portion of the associated project worksheet titled "Project 2: Population Collection and Description" was completed individually by each student upon the completion of this section of the lab 2 protocol.

2.5 Human Gene Investigation

Students began this lab by using the "Human Gene Description Worksheet Protocol." Using this protocol, students performed internet-based research and designed primers for their gene of interest. The lyophilized IDT primer tubes were spun down in microcentrifuges, then TE buffer was added to each primer (10 x nmol value; found on the IDT tube label; Figure 2.4). The primer tubes were briefly centrifuged and then placed in a 60ºC heat block for 60 minutes.

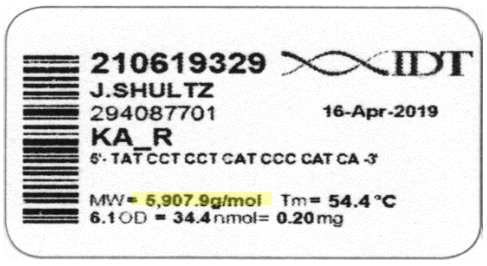


Figure 2.4. IDT primer tube label with nmol value highlighted.

The primer tubes were removed from the heat block and spun down. A 2.0mL tube was labeled using the stickers provided on IDT's primer description sheet. Next, 400uL of molecular biology grade water was pipetted into the labeled dilution tube. Then, 50uL of the left and 50uL of the right primer tubes was added to the labeled dilution tube. A 1.5mL tube was labeled with student initials; 30uL GoTaq and 12uL molecular biology grade water were added to the 1.5mL tube. Next, 6uL of the diluted primer pair was added to the 1.5mL tube and the tube was centrifuged. A strip of three PCR tubes, each tube containing 6uL of randomized human DNA, were labeled with student initials and numbers 1-3. Then, 15uL of the master mix was added to each of the 3 PCR tubes. The PCR tubes were spun down and then placed into a BioRad T100 Thermal Cycler using an initial denaturing step for 5 minutes at 95°, 35 cycles of denaturing for 60 seconds at 95°, annealing for 75 seconds at 50°, and extending for 45 seconds at 72°, and a final extending period for 5 minutes at 72°. All samples were held at 10ºC until removed from the thermal cycler.

After the PCR program was run, 10uL of each product was loaded into a separate well on an agarose gel, alongside a ladder. The gel was run for an hour to see if the samples worked by visualizing whether they produced a band which corresponded to each student's expected product size. A picture of the gel was taken using the UVP documentation system (BioDoc-It Imaging System, M-26). If a sample was successful, the remaining product from the PCR tube was loaded into a sequencing tube and sent for sequencing. Students performed a BLAST search on each sample to determine its identity. Upon completion of the Lab 3 protocol, students completed an oral presentation of their results.

2.6 Forensics

Students began this lab by documenting and photographing their own, unique crime scene. The lab instructor set up a diagram of a victim, upon which was an orange 2.0mL tube labeled with a suspect fingerprint letter (A-J) and a green 2.0mL tube labeled with a plant number, containing a plant leaf punch from the crime scene. Students were given a specific Suspect Fingerprint Sheet (Appendix B, Figure A1) which listed 12 CODIS primers and had a graphic showing the approximate band sizes of their suspect for each of the CODIS primers. In order to identify their suspect, students had to correctly interpret the information provided on their Suspect Fingerprint Sheet and compare their results to the "Human CODIS Fingerprints" database that was provided via an Excel sheet on Moodle.

Students used the protocol for plant DNA extraction (introduced in lab 3) in order to determine the location of their crime. Students extracted plant DNA, performed PCR, sent it for sequencing, then using the BLAST "Align 2 Sequences" feature to compare their samples to the plant sequences that that class had documented in lab module 3, students aligned their plant sequences and matched their plant sample to the "Louisiana Tech quad plant database" (Figure 2.3) to determine the location of their crime. Not all students received plant leaf punches from the mapped location. An oral presentation was completed individually by each student upon the completion of the lab 5 protocol.

CHAPTER 3

RESULTS

A total of five core and 12 optional labs were tested throughout this course. At the beginning of the course, students selected a trait of interest and a corresponding gene to focus on for the remainder of the quarter. The selected gene was used to complete a series of lab modules and oral presentations. Students were required to complete five protocols including prokaryote sequencing, human population DNA collection and description, plant population DNA collection and description, human gene investigation, and forensics. Once the required protocols were finished, students completed two (three for graduate students) more modules from a list of options. These prototype labs were in the rough development stage and included: allele migration, allele variation, gene structure, genetic counseling, natural selection, paternity testing, biochemistry, medical diagnosis, microbiology, parasitology, psychology, and pathology.

In total, 10 students (3 graduate and 7 undergraduate) completed the five required modules. Table 3.1 shows how many students selected and completed each optional lab module. Although microbiology, parasitology, and psychology modules were made available to students, no students selected those modules.

Table 3.1. List of Completed Lab Modules

Name of Lab Module	Number of Students
Required Labs	
Prokaryote Sequencing	10
Human DNA Collection and Description	8
Plant DNA Collection and Description	9
Human Gene Investigation	10
Forensics	10
Optional Labs	
Allele Migration	2
Allele Variation	7
Gene Structure	5
Genetic Counseling	6
Natural Selection	3
Paternity Testing	2
Biochemistry	3
Pathology	2
Medical Diagnosis	7
Microbiology	0
Parasitology	0
Psychology	0

3.1. Prokaryote Sequencing

During this lab, students collected bacterial samples from their mouth. A total of two types of media (LB and TSA) were used to grow the bacterial colonies. Students selected six colonies to identify via colony PCR. The colony PCR reactions used an ITS-M13 primer which targets an internally transcribed spacer (ITS).

A total of 54 samples were electrophoresed (Figure 3.1). Overall, 34 samples out of 54 had successful PCR and produced a ~180bp band. A total of 12 samples had too much bacterial DNA added to their PCR mix; these samples resulted in the appearance of smears during gel electrophoresis. Lastly, 8 samples failed to produce enough PCR product, which resulted in a faint band or absent band in the gel. Nine students completed this lab protocol over the course of two lab periods. One student was absent during the

first day of this experiment; this student's samples were not electrophoresed and were instead put directly into sequencing tubes after the PCR reaction had been run. Most of these reactions (44) were successfully identified using the 16S ribosomal RNA function of BLAST, with 10 reactions unable to be identified (Table 3.2). All six of the absent student's reactions resulted in successful sequencing. The most common genera were *Neisseria* and *Streptococcus*, with 11 matches each, followed by *Staphylococcus* with 7 matches.

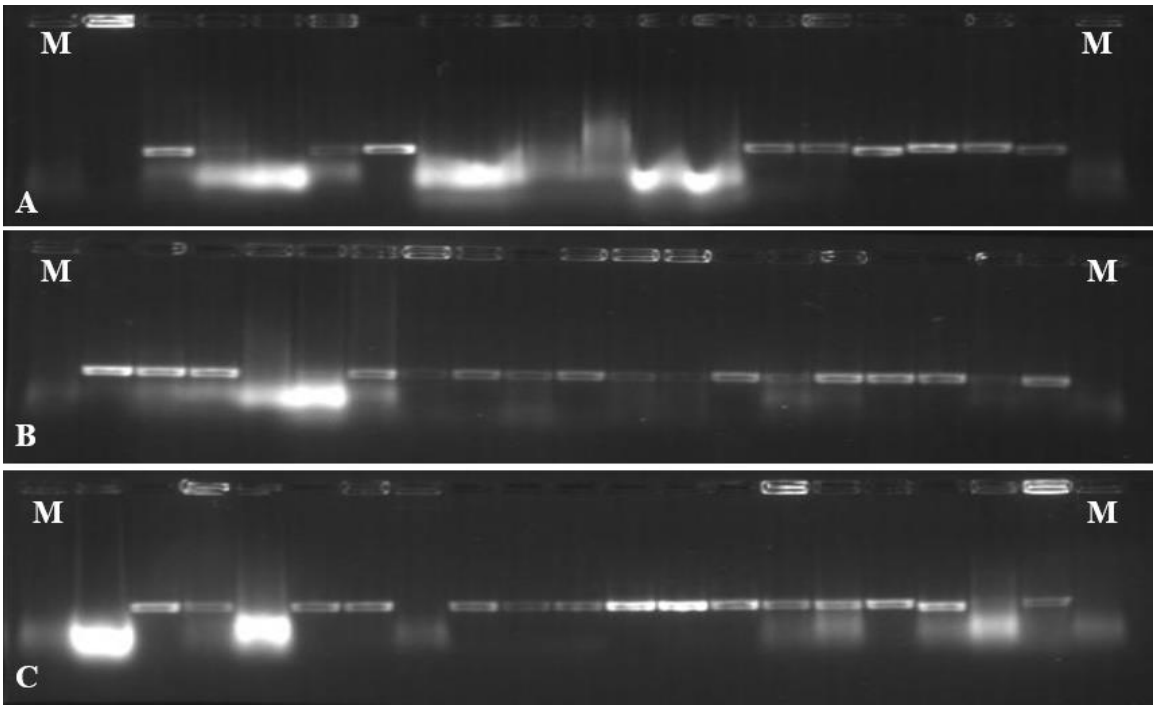


Figure 3.1. Gel electrophoresis of prokaryote colony PCR. All lanes have two failed markers (indicated by M). Panel A (samples 1-18), Panel B (samples 19-36), and Panel C (samples 37-54) contain a total of 34 working and 20 failed reactions.

Table 3.2. List of Identified Prokaryotes

Sequencing Tube Number	Identity of Sample	E-Value of Sample
MW5000	*Neisseria sicca*	7e-74
MW5004	*Rothia dentocariosa*	2e-61
MW5008	*Paenibacillus albidus*	9e-65
MW5009	*Staphylococcus epidermidis*	3e-71
MW5012	*Streptococcus oralis*	7e-73
MW5013	*Streptococcus salivarius*	5e-74
MW5016	*Neisseria sicca*	9e-72
MW5017	*Streptococcus oralis*	2e-73
MW5020	*Staphylococcus epidermidis*	2e-70
MW5024	*Neisseria subflava*	3e-69
MW5028	*Bacillus aryabhattai*	4e-78
MW3032	*Streptococcus sanguinis*	6e-74
MW5033	*Paenibacillus glycanilyticus*	4e-13
MW5036	*Neisseria flavescens*	7e-71
MW5037	*Streptococcus salivarius*	3e-69
MW5039	*Staphylococcus epidermidis*	6e-80
MW5040	*Gemella taiwanensis*	7e-58
MW5041	*Streptococcus salivarius*	3e-80
MW5044	*Neisseria flavescens*	1e-73
MW5045	*Corynebacterium singulare*	6e-61
MW5047	*Staphylococcus epidermidis*	1e-77
MW5049	*Paenibacillus etheri*	7e-75
MW5052	*Streptococcus salivarius*	3e-74
MW5053	*Neisseria flavescens*	3e-74
MW5055	*Staphylococcus epidermidis*	4e-77
MW5057	*Streptococcus salivarius*	4e-75
MW5061	*Neisseria perflava*	3e-70
MW5063	*Staphylococcus epidermidis*	1e-77
MW5065	*Paenibacillus etheri*	1e-76
MW5066	*Streptococcus oralis*	1e-68
MW5069	*Corynebacterium singulare*	7e-36
MW5071	*Staphylococcus epidermidis*	8e-70
MW5072	*Streptococcus oralis*	2e-71
MW5073	*Paenibacillus shirakamiensis*	2e-61
MW5074	*Paenibacillus etheri*	9e-75
MW5076	*Neisseria flavescens*	4e-73
MW5077	*Neisseria flavescens*	9e-70
MW5079	*Staphylococcus epidermidis*	1e-78
MW5080	*Streptococcus oralis*	3e-75
MW5081	*Massilia consociata*	9e-25
MW5082	*Streptococcus oralis*	3e-80
MW5084	*Neisseria perflava*	1e-75
MW5085	*Neisseria flavescens*	3e-72
MW5090	*Paenibacillus etheri*	1e-73

3.2. Human Population Collection and Description

This project consisted of two portions: human DNA extraction and collection, and human fingerprinting. Students utilized Instagene Matrix (Bio-Rad, cat. # 732-6030) and a combination of centrifuge and incubation steps to extract their own DNA. As shown in Figure 3.2, students performed human fingerprinting using randomized DNA samples and a total of 14 CODIS primers (Table 2.1).

A total of eight students completed the entirety of this experiment over the course of two lab periods. On day one of this experiment, human DNA extractions were performed and the PCR reactions for human fingerprinting were prepared and amplified; two students missed this lab period. On day two of this experiment, the human fingerprinting PCR reactions were loaded into a gel, electrophoresed, and documented; all students were present this day. The two students who missed day one of this experiment were unable to participate in the second portion of this experiment. DNA extraction success was confirmed by the PCR product.

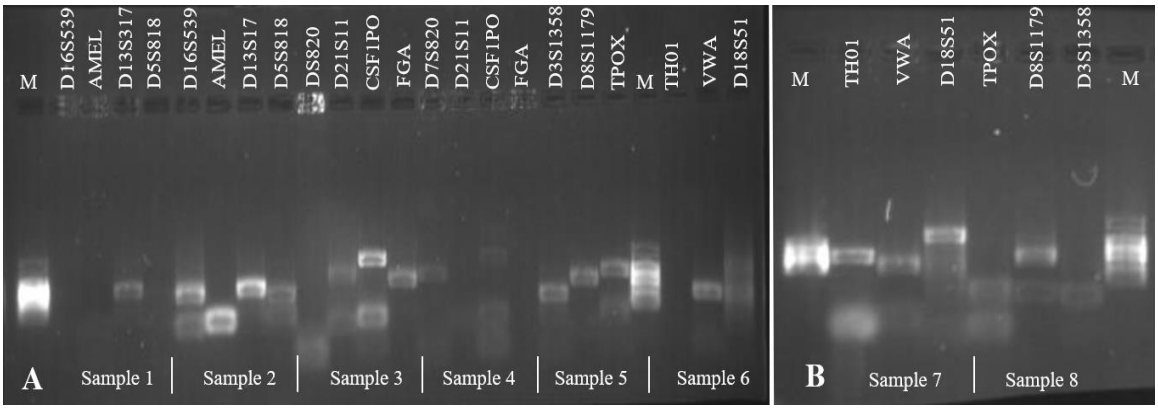


Figure 3.2. Gel electrophoresis of human fingerprinting. All marker lanes failed (M). All samples were randomized human DNA. Panel A shows samples 1-6; panel B shows samples 7 and 8. As shown in Figure 3.2, 5 of 8 samples had successful PCR results and produced one or two bands for all primers. A total of 3 samples failed to produce an acceptable amount of PCR product and resulted in a faint band, an absent band, a smear, or a combination of both for at least one of the primers tested.

3.3. Plant Population Collection and Description

During this lab, students collected plant leaf punch samples from the quad area of Louisiana Tech University's campus. The location of each plant was recorded on a map of the quad area (Figure 2.3). Students performed DNA extractions on them, made PCR reactions, ran the reactions on a gel, and sent the successful samples out for sequencing.

A total of nine students completed the entirety of this experiment over the course of three lab periods. On day one of this experiment, plant leaf samples were collected, and DNA extractions were performed; one student was absent on this day. On day two, PCR reactions were prepared and amplified. On day three of this experiment, the PCR reactions containing plant DNA were loaded into a gel, electrophoresed, and documented; all students were present this day. The student who missed day one of this experiment was able to participate in the second portion of this experiment.

A total of 29 samples were electrophoresed. Overall, 27 samples out of 29 had successful PCR results and produced either a ~500bp band or a ~750bp band, depending on the primer combination used. All of the reactions loaded into gel #1 (except for the sample in well #7, it used the U1-U4 primer combination) were made using the forward primer P3 and the reverse primer U4; the expected product size for this primer combination was ~500bp. All of the reactions loaded into gel #2 were made using the forward primer U1 and the reverse primer U4; the expected product size for this primer combination was ~750bp.

Ultimately, two samples failed to produce enough PCR product, which resulted in either a faint or absent band on the gel. Most of the plants (26) were successfully

identified using BLAST analysis (Appendix C, Table A2). A total of three reactions were unidentifiable via BLAST.

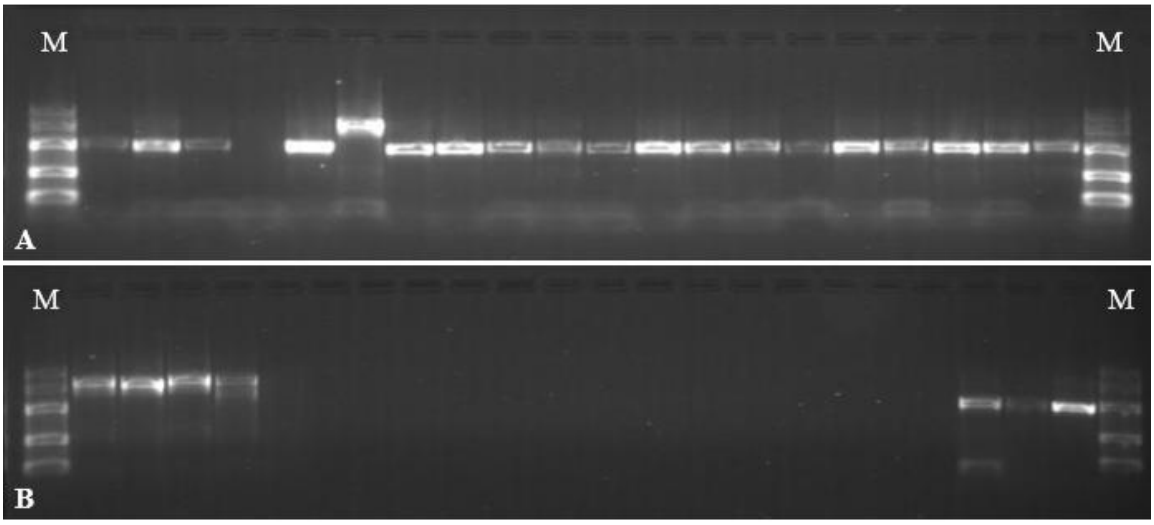


Figure 3.3. Gel electrophoresis of plant DNA. Wells containing size standards are labeled with "M." In panel A, samples 1-5 and 7-20 used the P3-U4 primer combination. Well 5 of panel A and all wells on panel B used the U1-U4 primer combination.

3.4. Human Gene Investigation

During this lab, students selected a trait of interest and a corresponding gene to investigate. Students presented their research and results from this lab as part of their last oral presentation. All 10 students completed this lab over the course of several lab days.

Overall, 33 samples were loaded into the gel but two of these were accidental repeats of samples and one was a ladder loaded on accident (Figure 3.4). In total, 30 samples produced bright, acceptable bands on the gel, but only 20 of these resulted in successful BLAST results. A total of 10 reactions (of the 30) failed to produce successful BLAST results. Of the 10 primers tested, nine of them resulted in at least one successful BLAST result. Due to size standard issues, a commercial ladder was used for this gel.

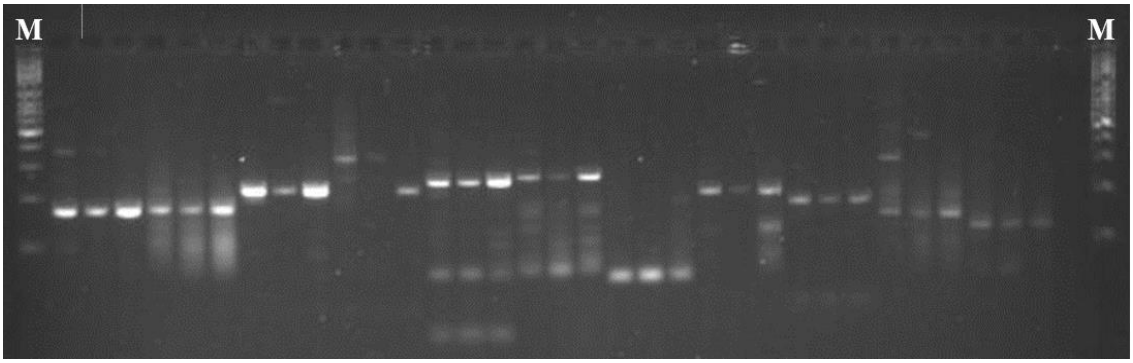


Figure 3.4. Gel electrophoresis of human genes. Wells containing size standards are labeled with "M." In total, 33 samples were electrophoresed, but only 30 samples were analyzed using NCBI BLAST. Of these 30 samples, 20 samples produced the expected band size and produced BLAST results which showed a significant match to the correct target chromosome and/or gene location. Six of the samples produced the correct band size but failed to produce expected BLAST results (3 samples produced BLAST results which showed a match to an off-target chromosome; 3 samples resulted in non-human BLAST results). Lastly, the PCR reactions for 4 samples failed, which resulted in bands of the wrong size; these samples failed to produce BLAST results.

3.5. Forensics

During this lab, students had to rely on skills learned throughout this course to solve a crime case. Each student had a unique case to investigate. Briefly, each student documented their crime scene, determined their suspect, and determined where their crime took place.

All 10 students completed this lab over the course of several lab periods. All students correctly identified their suspect using the backup, digital "CODIS Fingerprint Database." Four students' plant BLAST results worked correctly, and the students were able to determine the location of their crime. Six students' plant BLAST results failed to work correctly (they matched with a portion of *all* of the plant sequences in the database) and the students were unable to determine the location of their crime.

There were two types of BLAST results for this lab; (1) a short sequence which matched to all of the sequences in our plant database (with an e-value ≈ 4e-56) and (2) a

more complete match to a specific plant in our plant database with a substantially higher e-value, typically 3e-172 (Figure 3.5).

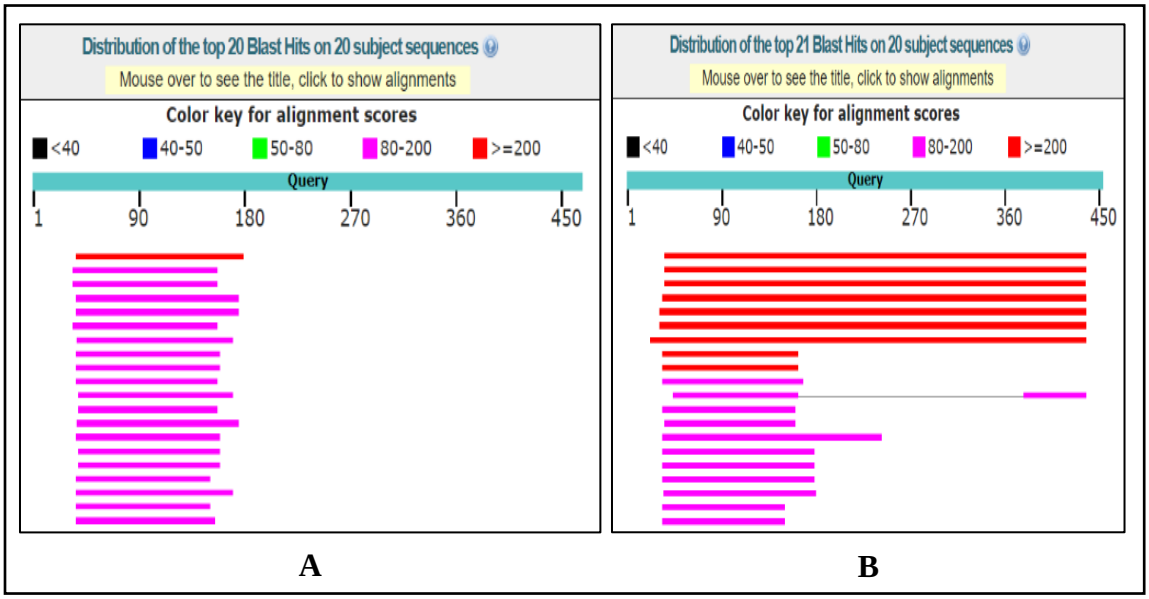


Figure 3.5. The two types of plant BLAST results for the forensics lab protocol. Panel A shows the BLAST results when a short sequence of a sample matched to all of the sequences in our "plant database." Panel B shows the BLAST results when a more complete match to a specific plant in our "plant database" with a significantly higher e-value.

3.6. Optional Labs

Two optional labs had interesting results. Allele migration required the placement of reported alleles on a human migration map (Figure 3.6). Paternity testing posed the question as to whether a single gene could be used to determine paternity, requiring substantial single nucleotide polymorphism (SNP) analysis to determine the most likely vs. the least likely inherited genotype of an individual (Figure 3.7).

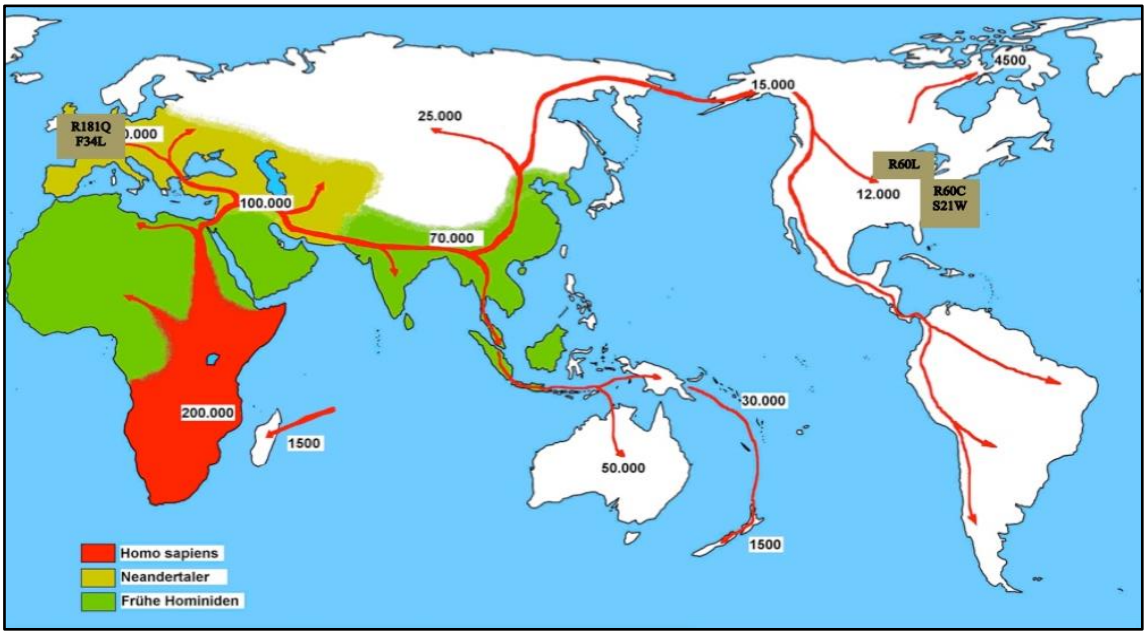


Figure 3.6. Example of reported GNA11 disease alleles on a human migration map (map from Wikipedia sources [McEvedy & Jones, 1978; Thomlinson, 1975]).

Identify the representation of the molecular markers (SNPs) in the fragment (use at least 10 markers from this gene)

SNP/InDel	Chr 19 Position	Alleles	Freq %
rs145870964	3,113,519	+/15bp del	0.953/0.047
rs6510733	3,113,579	C/T	0.986/0.013
rs7258655	3,113,772	T/C	0.796/0.203
rs74380612	3,113,782	C/T	0.847/0.152
rs17749145	3,113,841	A/G	0.890/0.109
rs72975292	3,113,869	C/G	0.982/0.179
rs385443	3,113,890	C/G	0.562/0.437
rs411386	3,113,905	G/T	0.565/0.434
rs113732637	3,114,161	C/T	0.976/0.023
rs72975294	3,114,179	G/T	0.982/0.017
rs3786947	3,114,231	C/T	0.779/0.220
rs3786946	3,114,287	G/A	0.857/0.142
rs80081057	3,114,416	C/T	0.976/0.023
rs404632	3,114,547	C/T	0.742/0.257
rs57763090	3,114,590	G/A	0.983/0.016
rs115640077	3,114,703	C/T	0.955/0.041
rs3746069	3,114,866	G/A	0.830/0.169

Identify how accurate sequencing just your gene would be in determining paternity.

Most Common	homozygous	Likelihood	Least Common	homozygous	Likelihood
0.986	0.972196	0.972196	0.013	0.000169	0.000169
0.796	0.633616	0.615998941	0.203	0.041209	6.96432E-06
0.847	0.717409	0.441923184	0.152	0.023104	1.60904E-07
0.89	0.7921	0.350047354	0.109	0.011881	1.9117E-09
0.982	0.964324	0.337559065	0.179	0.032041	6.12527E-11
0.562	0.315844	0.106616005	0.437	0.190969	1.16974E-11
0.565	0.319225	0.034034494	0.434	0.188356	2.20327E-12
0.976	0.952576	0.032420442	0.023	0.000529	1.16553E-15
0.982	0.964324	0.031263811	0.017	0.000289	3.36838E-19
0.779	0.606841	0.018972162	0.22	0.0484	1.6303E-20
0.857	0.734449	0.013934086	0.142	0.020164	3.28733E-22
0.976	0.952576	0.013273275	0.023	0.000529	1.739E-25
0.742	0.550564	0.007307788	0.257	0.066049	1.14859E-26
0.983	0.966289	0.007061435	0.016	0.000256	2.94039E-30
0.955	0.912025	0.006440205	0.041	0.001681	4.94279E-33
0.83	0.6889	0.004436657	0.169	0.028561	1.41171E-34

Figure 3.7. Example of SNP analysis for paternity testing.

CHAPTER 4

DISCUSSION

This project had multiple goals. The first task was to assess the initial development of laboratory exercises that can be independently selected and performed. Second, each completed lab protocol was evaluated to assess how well the protocol teaches students the core concepts of genetics which include: the Central Dogma, Transmission Genetics, and Population Genetics. The third task was to analyze whether each of the protocols are robust enough to stand up to students with a basic skill set, and if so, to assess how each protocol improves this basic skill set. Finally, the method of assessing student success and knowledge gained from each of the lab protocols was established.

Although labs were initially selected by students and unique student manuals were produced, it became clear that with only ~100 pages of protocols (50 pages front and back), simply printing all protocols in a single book would be the simplest solution. This allows students to select from the included labs and removes the problem of printing a unique book for each student.

Every required lab used in this class failed to directly indicate the relationship of the Central Dogma, Transmission Genetics, and the Hardy-Weinberg assumptions to the work performed. This direct linkage is being added to the next version of these course materials.

Each student that registered for this class had the basic skill set required for completion of the lab exercises. This was due to their previous experience in PCR Methods, the required prerequisite course. Most students who have taken Genetics would have the technical skill set required but would have to learn the research skills necessary to apply their technical capabilities.

4.1 Required Labs

A total of 17 labs were tested throughout this course. Students were required to complete five modules including prokaryote sequencing, human population DNA collection and description, plant population DNA collection and description, human gene investigation, and forensics.

4.1.1. Prokaryote Sequencing

During this lab, students collected bacterial samples from their mouth. Two types of media (LB and TSA) were used to grow bacterial colonies. Students selected six colonies to observe and identify via colony PCR. The colony PCR reactions used an ITS-M13 primer which targets an internally transcribed spacer (ITS).

ITS sequences are located in the DNA which encodes part of the ribosomes found in prokaryotes. ITS primers utilize highly conserved ribosomal encoding sequences to design PCR primers which flank highly diverged ITS sequences. Since this region is highly conserved, the PCR reactions have an excellent chance of working correctly. This allows bacterial samples to be identified based on their highly diverged ITS sequences via BLAST analysis [DeCaire et al., 2015].

One broad goal of this lab was to allow students to get familiar with common techniques and procedures which will be utilized in further lab protocols. These

techniques included: basic microscope skills, pipetting, setting up a PCR reaction, loading a gel, sending samples for sequencing, and using NCBI BLAST for sequence analysis. For example, students who were not well versed in how to use a pipettor or prepare a master mix had an opportunity to learn these skills at their own pace, while students who had already mastered these skills had an opportunity to acquaint themselves with these procedures once again.

Another goal of this project was to teach students about the human oral microbiome while emphasizing common techniques used in molecular genetics. Usually, prior to taking a genetics course, the only knowledge a student has of the human oral microbiome is what they learn in a microbiology course. A major component of microbiology courses is teaching students how to identify bacteria based on the results of biochemical tests. In this advanced genetics course, students are taught how to identify bacteria using colony PCR and sequencing.

This lab was perhaps the most complete in terms of protocol, achievement of expected results, and assessment (via a worksheet and a presentation). This was due to the protocol being an extension of a well understood, practiced protocol with years of execution in Genetics and PCR laboratories.

Since both Genetics and PCR Methods were prerequisites for this course, the majority of students were familiar with the procedure used for this lab. However, this lab protocol was adapted to be more complex than it was when utilized in other courses. For example, this is the first time that different agar types were used for this experiment. Additionally, this is the first time that bacterial colony morphology (with the use of a dissecting microscope) was included as part of this laboratory protocol. Students had to

use a microscope to count and classify all of their bacterial colonies carefully. The most common student concerns were about having to describe all colony morphology found on their plates and having to generate educational questions as part of the worksheet that went along with this protocol.

A major point of this protocol is that students were *not* clearly instructed to have a standard reason for selecting and sequencing certain bacterial colonies. As a result, some students randomly selected bacterial colonies. This led to multiple students having to create (or fabricate in some cases) the reasoning for selecting theirs. In future courses, it should be stressed to students to have a scientific reason for selecting and sequencing their bacterial colonies before the wet-lab component of this protocol is performed.

An additional aspect of this protocol was not teaching students how to use BLAST to analyze their samples. Some students relied on the "percent match" analysis generated by BLAST rather than relying on the "e-value" of their sample because they did not understand that "percent match" is not a statistical measure. This was probably because students were not instructed how to use BLAST for the assignments associated with this protocol. This represents an opportunity to introduce the subsequent Central Dogma and the idea of conservation into project questions.

The protocol for this lab successfully promoted independent thinking and personal responsibility. Students quickly realized that it was entirely their responsibility to perform all aspects of this lab. Students had to express their own reasoning and unique results for this lab when completing the assignments for this protocol; relying on others to do the work for them was not a viable option.

According to Figure 3.1, approximately 63% (34 of 54) of the samples were successfully amplified and produced a ~180bp band. A total of 20 samples were not successfully amplified; 12 of these samples had too much bacterial DNA added to them, and produced a smear on the gel; eight samples did not contain enough product and resulted in a faint or absent band.

As shown in Table 3.2, most samples (44 of 54) were identified via BLAST (~81%). This was surprising because only 34 samples produced successful PCR results, indicating that half of the samples which appeared to fail PCR contained enough bacterial DNA to be identified using BLAST.

This lab was flexible in terms of student absences. If students missed a lab day, they could easily make up the missed lab work when they came back. For example, one student was absent on the day that gel electrophoresis was performed. This student's samples were not electrophoresed and were instead put directly into sequencing tubes after the PCR reaction had been run. These samples still produced successful sequencing results. Flexibility in the timing of lab procedures should be a goal of the protocols in this study and ties in directly to independence; the lab instructor does not have to allot time to make sure all of the students are performing at the same pace, and the student does not have to stress if missing a lab day is necessary.

4.1.2. Human Population Collection and Description

This project consisted of two portions: Human DNA collection and fingerprinting using CODIS primers. This lab was designed to collect human DNA and then accurately describe it so that it could be used during the Forensics protocol. A broad goal of this lab was to teach students how to perform a human DNA extraction, and introduce students to

analyzing human DNA samples by using the CODIS primers which the FBI uses to identify individuals [Budowle et al., 2001; Eng, Ainsworth, & Waye, 1994; National Institue of Science and Technology, 2019].

CODIS primers function by utilizing short tandem repeats (STRs). STRs are short, repetitive sequences of DNA that are 1-6 bp long [Fan & Chu, 2017]. These fragments are inherited in a Mendelian fashion and have high levels of variance, which means that they are unique from person to person and can easily be used to identify the parents of an individual. Because of these features, STRs are utilized in forensics to identify suspects and to perform paternity tests.

This lab was not complete in terms of protocol, achievement of expected results, and assessment. Certain aspects of this protocol worked well, such as the human DNA extraction procedure; however, other aspects of this protocol require adjustment, such as the fingerprinting protocol.

The human DNA collection portion of this lab worked well. Again, students are exposed to this process during both Genetics lab and PCR Methods lab, so most students were comfortable and familiar with this section of the protocol.

Describing human DNA via fingerprinting with CODIS primers was unexpectedly difficult. Overall, approximately 71% (20 of 28) of samples failed to produce useable results. As seen in Figure 3.2, many of the samples produced unclear, fuzzy-looking bands which were difficult to interpret. Some samples (6 of 28) failed to produce any PCR product during this procedure, while others (14 of 28) produced a faint band and/or a smear. Although approximately 29% of samples (8 of 28) produced acceptable results, this was not enough samples to be utilized for the Forensics protocol.

This lab did briefly expose students to Transmission Genetics and one Hardy-Weinberg assumption. The connection between STRs and Transmission Genetics was not directly indicated, so some students did not make the connection between these two topics. The one Hardy-Weinberg assumption that was addressed was Mutation. As with the STRs, the connection between these two topics was not clearly addressed, so students failed to make this connection.

The protocol for this lab did successfully promote personal responsibility. Students were responsible for extracting their own DNA and preparing their own PCR reactions. This lab did not connect the DNA collection and CODIS fingerprinting directly, which will need to be addressed in the next revision of the lab manual.

This protocol was not as forgiving in terms of student absences as the previous lab. Two students were absent the day that human DNA extractions were performed, and could not make up this portion of the protocol. Because of this, the two students who missed the previous lab session were unable to participate in the second portion of this experiment when human fingerprinting PCR reactions were run in a gel. This protocol must be executed in a more independent manner to be effective.

4.1.3 Plant DNA Collection and Description

During this lab, students collected plant leaf punch samples, performed DNA extractions on them, made PCR reactions, ran the reactions on a gel, and sent the successful samples out for sequencing. The PCR reactions used an ITS-M13 primer which targets an internally transcribed spacer (ITS). These spacers are described in section 4.1.1, as they relate to bacterial DNA. The procedure for this lab utilizes similar techniques and concepts, but with PCR primer specific to plant DNA.

Two goals of this lab were to inform students how to perform basic plant DNA extractions and to test whether sequencing could be utilized to correctly identify various plant species. The initial plan for this lab was to have it function similar to Lab 2 (Human DNA Collection and Description), using CODIS primers to describe plant DNA. However, since using CODIS primers failed in the previous protocol, the protocol for this lab was adapted to attempt identification of plants via sequencing instead.

Another goal of this lab was to generate a map of plant locations within the quad of Louisiana Tech. This map was utilized during Lab 5 (Forensics). There were some plants with the same appearance which were indicated in multiple locations on the map. The sequencing for these plants indicated that they were the same species, but did have slightly differing sequences from one another.

This lab worked far better than expected. The sequencing of plant DNA had not been performed before, yet the samples had a high success rate (~93%). Not only did the sequencing work, but the Google search of the named species typically yielded a close visual match to the plant pictures taken by the student. In total, 29 plant DNA samples were electrophoresed (Figure 3.3). However, some of these samples were amplified using both ITS-plant primer combinations (Table 2.2); of these 29 samples, 27 samples had successful PCR results (~93% of samples tested worked). As shown in Figure 3.3, two PCR reactions failed to produce enough product. Overall, 22 plants were analyzed utilizing BLAST and 19 of these plants were successfully identified (86% success rate).

A considerable problem with this protocol is that it failed to integrate core concepts of genetics (the Central Dogma, Transmission Genetics, and Population Genetics) into this experiment. Students did receive exposure to the Scientific Method,

but this was not stressed to students. Thus, some students failed to connect this protocol to that concept.

This protocol was successful at teaching personal responsibility. Students were not given explicit instructions on how to go about dividing the workload of mapping plants in the quad, but all students worked together to problem-solve. Students ended up dividing the quad into four sections and then assigning groups of 2-3 students to collect plant samples from a specific quadrant. This method worked well and promoted the idea of personal responsibility because if a group of students failed to collect and catalog a plant, other students' research would likely be affected.

This protocol was also successful at promoting independent thinking through the assessment (a worksheet and oral presentation) that went along with this lab. Part of each student's presentation required an explanation of their sequencing results and BLAST analysis, which drove students to think on their own and carefully assess their data. Students had to learn how to correctly interpret and explain the results of the chromatograms for each of their samples.

This lab was flexible in terms of student absences. Section 2.3 outlines the days that each component of this experiment occurred. One student was absent on day one of this protocol and did not get to make up this portion of the lab. This student was still able to participate on days two and three of this experiment. However, this student did not have the opportunity to complete the oral presentation component of this protocol because the presentation relied on students gathering their own plant samples on day one. This student only completed the worksheet associated with this protocol as a form of assessment for this lab.

4.1.4 Human Gene Investigation

During this lab, students selected a trait of interest and a corresponding gene to research and design primers for. The information gained during this protocol helped students complete some aspects of the optional labs that they chose.

This lab was mostly complete in terms of protocol, achievement of expected results, and assessment (initially via a worksheet and a presentation). This was due to the protocol being an adaptation of two well-understood protocols with years of execution in the Genetics lab. Parts of this protocol were identical to a well-established procedure utilized in Genetics, a prerequisite for this course.

Another major goal of this protocol was to teach students how to design primers for a gene of interest. Students were also familiar with part of the protocol that was used to complete the primer design aspect of this lab. These primers were then tested for functionality using three anonymous samples of human DNA (Figure 3.4). Of the 10 primers tested, nine of them resulted in at least one successful BLAST result. Of the 30 electrophoresed reactions, 20 of them resulted in sequences that were associated with the correct target gene (66% success rate), which is approximately equal to previously reported primer design success [Shultz, 2008].

This protocol did integrate the Central Dogma, Transmission Genetics, and Population Genetics, but in a less direct way than desired. One requirement for students' final presentation was that they establish the significance of their gene of interest. This successfully integrated the Central Dogma into this protocol. An additional requirement of students' final presentation was an explanation of the phenotype of their disease of interest, which resulted in the integration of Transmission Genetics into this protocol.

The protocol for Lab 4 successfully promoted independent thinking and personal responsibility. Students quickly realized that it was their responsibility to perform all aspects of this protocol correctly. For example, if a student failed to address the significance of their gene of interest during their final presentation, other students would call them out on missing information. This directly correlated to their grade on the final presentation. Additionally, students had to express and explain their own reasoning and unique results for this lab when completing the presentation for this protocol.

Since most of the work that went into completing this protocol was independent, out-of-lab work, student absences did not affect the completion of this protocol. The only way for a student to become behind on this is if they failed to submit their primers in time for ordering with the rest of the class. None of the students did this, but if this were to occur, the primers of that student could be ordered at a later time.

4.1.5. Forensics

During this lab, each student had to investigate a crime scene. Students were required to document their crime scene, determine their suspect, and determine where their crime took place. This protocol and subsequent lab proved to be the most problematic of all.

Initially, this protocol was going to serve as the cornerstone project for this course. Students would use the skills and techniques they had learned throughout the quarter to prove their case. Human fingerprinting using the CODIS primers proved unrepeatable. The solution to this was to fabricate a “CODIS Fingerprint Database” that contained 153 unique “CODIS fingerprints.” Students were each given a Suspect Fingerprint Sheet (Appendix B, Figure A1) and told to identify their suspect by

comparing their sheet to the CODIS Fingerprint Database. As a result of this, the suspect identification portion of this protocol was extremely oversimplified. This procedure had been utilized before and was a backup to wet lab fingerprinting, but was not the desired protocol.

The plant analysis aspect of this protocol managed to both work and fail at the same time. Students were given a 2.0mL tube containing a plant leaf punch and instructed to perform plant DNA extraction. All students successfully extracted, amplified, and sent their plant DNA for sequencing. Students were then instructed to align their plant sequence (using a function of BLAST) with the sequences in the class "plant database." There were two types of BLAST results for this lab: (1) a short sequence which matched to all of the sequences in the plant database; and (2) a more complete match to a specific plant in the plant database with a significantly higher e-value (Figure 3.5). Four students' plant BLAST results worked correctly, and the students were able to determine the location of their crime via correlating their plant sequence with the location of the plant on the generated plant map of the quad. Six students' plant BLAST results matched with a portion all of the plant sequences in the database and the students were unable to determine the location of their crime.

An additional problem with this lab is that the core concepts of genetics (the Central Dogma, Transmission Genetics, and Population Genetics) were not clearly identified in this protocol. This protocol did promote personal responsibility since each student had a unique case to analyze and attempt to prove. Students had to rely on their own understanding of each of the adapted procedures to complete the required presentation for this lab.

4.2. Optional Labs

Optional labs were initially created to allow students flexibility in selecting their research interest in their gene. The desired outcome of this was for students to form a unique perspective on their gene and trait of interest. Their task was then to create oral presentations that would allow them to discuss what they had found. A final presentation which incorporated all of a student's findings from the optional labs, as well as their findings from Labs 4 and 5, was created instead. These optional labs were designed to reinforce specific Hardy-Weinberg, Transmission Genetics and Central Dogma concepts and were direct applications of these concepts.

4.2.1. Allele Migration

For this lab, students identified human migration and segregation patterns for their gene of interest. Students had to find 3-6 locations from around the world where specific alleles for their gene of interest were unique to specific human populations. Students had to add this information to a map of human migration patterns to show how and where their gene of interest changed over time.

4.2.2. Allele Variation

For this lab, students identified variations for their gene of interest. These variations had to include variations in the coding sequence as well as variations in the produced polypeptide. Students had to use the University of California Santa Cruz (UCSC) Genome Browser to identify the number of SNPs and the number introns and exons within their gene of interest. Students also had to identify which genomes of other organisms shared a significant portion of the sequence for their gene of interest with humans.

4.2.3. Gene Structure

For this lab, students identified the gene structure for their gene of interest. Students had to use the UCSC Genome Browser to identify and show a picture of the number of introns and exons within their gene of interest.

4.2.4. Genetic Counseling

For this lab, students identified the inheritance pattern for their gene of interest. Students were to use the Online Mendelian Inheritance in Man (OMIM) website to find this information. Students were to discuss how the inheritance pattern could potentially affect the children of individuals carrying or affected by their disease of interest. Students also had to discuss a relevant pedigree for this protocol.

4.2.5. Natural Selection

For this lab, students identified the potential benefits for their gene of interest. Students were to use OMIM or the MalaCards database to find this information. Students were to discuss how the possible changes to their gene of interest could increase chances of survival, and subsequently lead to the prevalence of individuals affected with (or carrying) their disease of interest.

4.2.6. Paternity Testing

For this lab, students identified sequence variations within ~600bp fragment of their gene of interest which could be used for paternity testing instead of (or in addition to) the CODIS primers. Students had to use the UCSC Genome Browser to identify and show a picture of this variance within their gene of interest. Students also had to analyze the rates of hetero- and homozygosity of their gene to determine how accurate sequencing their gene would be in determining paternity.

4.2.7. Biochemistry

For this application-based protocol, students investigated the folding pattern for the protein produced by their gene of interest. Students were told to identify the domains, chemical properties, pathways, and receptors for this protein. Students were also told to discuss what happens when their protein stops working correctly.

4.2.8. Medical Diagnosis

For this lab, students identified medical or genetic tests that are available to test for their disease of interest. Students were also told to discuss in detail any possible treatment options available to individuals affected with their disease. Students also had to include a graphic of the diagnostic tests available.

4.2.9. Pathology

For this lab, students identified tissue-specific expression patterns for their gene of interest. Students were told to include why and how the most common disease phenotype is caused by their gene.

CHAPTER 5

CONCLUSIONS

This project was deceptively complex. Although it was understood that there were a large number of moving parts (this was why the class was limited in size), it rapidly lost cohesion during execution. There were many parts that worked, but there were just as many parts that did not.

The independent selection of labs and creation of unique lab manuals was far less difficult than it sounds, but higher numbers of students will render this portion of the project unnecessary. To put it simply, all of the core labs and optional labs currently occupy less than 100 pages (or 50 pages of actual paper). This small manual can be printed for everyone, making preparation for class far less complicated. The student still selects their gene, but receives a full-size manual and performs only selected labs.

The independent execution of labs was a problem at two levels. We had planned for independent execution (no group projects) but had not accounted for the *class* as a group. By utilizing core required labs, we simply replaced a student group leader with the instructor. This affected all levels of execution through most of the course; it tied all students to the instructor and to each other. The next iteration of this project will remove the instructor as the "leader" and allow nearly full autonomy.

The incorporation of the core concepts of genetics was very uneven. Each of the five required labs were exceptionally poor at directly indicating which of these concepts

were important to the task at hand. Many of the optional labs were exceptionally good at this, driving home these ideas directly. Because of the variable nature of gene selection and optional lab selection (also tied into the gene selected), there is no possibility of creating a grading "key" for this course. With limited practice, however, it is almost faster to grade these exercises; they include requirements of evidence to be placed into the answer. Therefore, once the instructor is aware of what the evidence looks like, grading is very easy. The class need to tie in the core concepts with each lab is easily addressed by changing the assessments at the end of each project.

Given the positive and negative aspects of this study, the next steps are obvious: improve the reliability of all labs, especially required ones; remove instructor input and control wherever safely possible and incorporate the core concepts directly into all of the lab exercises.

APPENDIX A

PLANT SAMPLES SEQUENCE LIST

Table A1. Plant Samples Sequence List

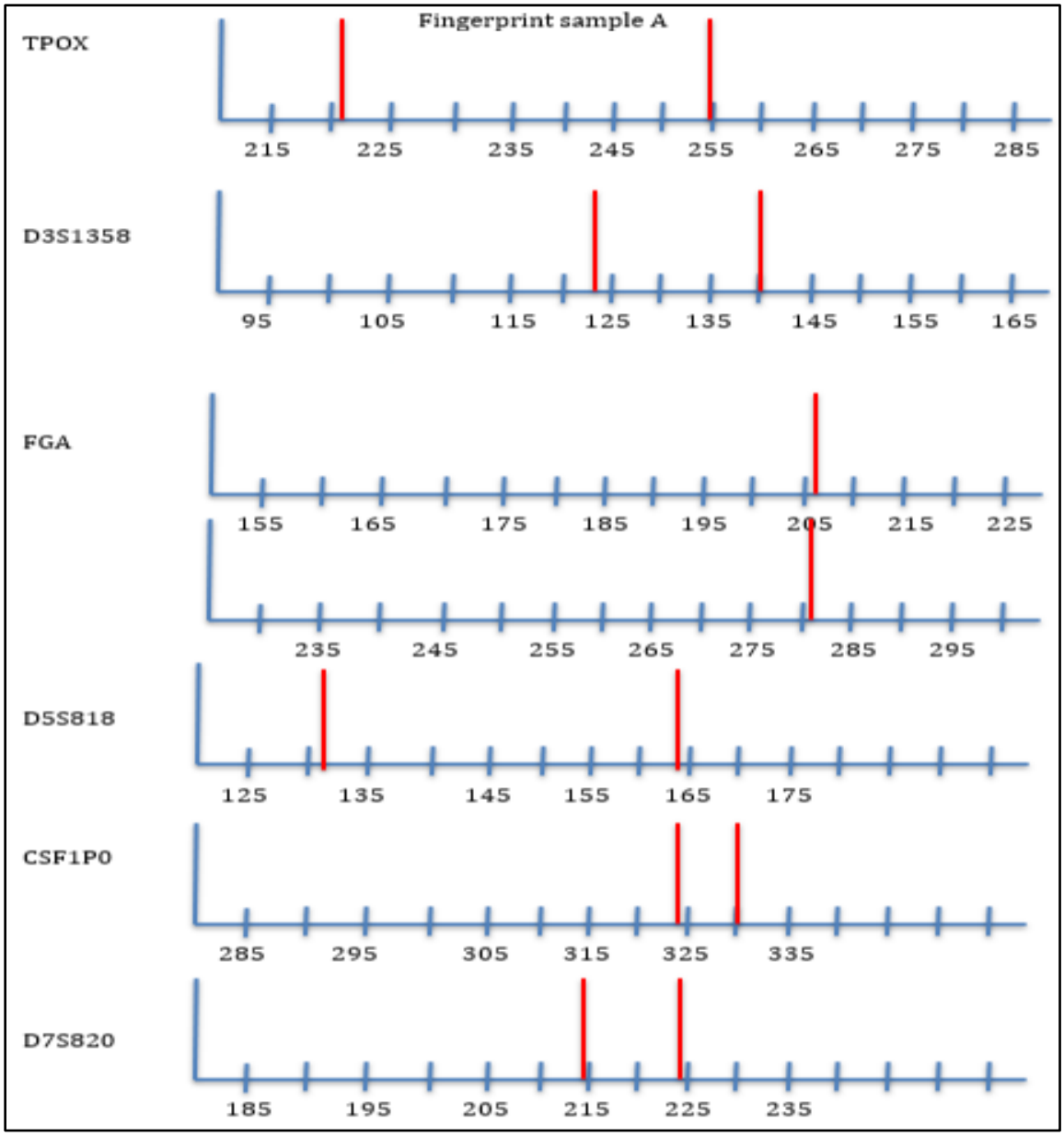
Plant Sample Number	Plant Sequence
0	NNNNNNNNNNGGNTNNNGNATCNNNGNNACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAAT CCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGCCGAGGGCACGTCTGCC TGGGCGTCACACGTCGTTGCCCCCCCCCAACCCCTNNGGANNTGGANGGGNNNGANGAAGGCCNNCC GNNNNCNCCNTCCCCCCGTTTGNATNNAAACCNAGGGCCCNGGGACNNAANNCCCNNNNAANGGGG GGTNTNNNANNNCCNGTTNNNNNNNNGNGCNCNNNNNNNNNANNGNTTNNTNNAAANNNNGNGNN NNNNCNNCNATTTNTTTANCCCNNCCCCNGGGGGGGGGGGNNCNNNNNNTTTTNNNNNNNNNNNGN GGAAAAAAAAAAAAAAAAN
1	NNNNNNNNNNAGGCTCTCGCATCNATGANNANGTAGCAACTGTGATACTTGGTGTGAATTGCAGAAT CCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGCCAAGGGCACGTCTGCC TGGGCGTCACGCATCGCGTCGCCCCCCCCACCCCAGTGGTGTCGGGGCGGAAAATGGCATCCCGTGGT CTGTCGCGGCCTGCCTAAACCCGTGTCCCTCGTCGCGGACGTCACGACAAGTGGTGGTTGAAATCCTC AACTCGAATGCGAGTCGTGCGCACCTCGNGGCCGAGACGACACGTAATAGACCCTTAGACGATTCCC TTTCNAGGGAGGAGCATACNTCATGACTGCAACCCCNGGTNAGGCGGGGCTACCCGCTGAGTTTAAG CATATCANTAANCGGNNNNNNANAAACTN
2	NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNACGTAGCGAAATGCGATACTTGGTGTGAATT GCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCAAAGCCATTAGGCTAAGGGCAC GTCTGCCTGGGCGTCACGCATCACGTCGCCCCCAACCCCAAATGCCTTGATGTTGCGGGAGTTGGGGG CGGAAATTGGCCTCCCGTCCACACGACCGTGAGCGGTTGGCCCAAAAAATGAGTTCCTGACGAAGGA CGTCACGACAAGTGGTGGTTGAAAGACCTCTTGCATCATGTCGTGAGGCGCCTAGTCTGTAGCGAGCT CTAACCGTGGACCCTGCGCACCTAATTCGTTCCCGAAGGANAGCGACGACGGTGCTTCGACCGCGAC CCCCGGTCAGACGGGATTACCCGCTGAGTTTNAGCATATCAATNAGCGGAANAAAAAAAANNAN
3	AGCACTAGGCTTAGCAAGTACTTTATTGCCATGGACATATATTGTAGAGAGAAAATAAGAAGAAATA AAATTGAACAATGAGTAAGCGGAGGAAAAGAAACACCCTGCGTCCCCCTGCGGGCCCGTAATAGGA AACGAACCCCCGGCGCTGTCTGCGCCAAGGAACCATAACCGAGAGCTGGCCTCCCGATGTCCCGTGC GCGGTGCGCACGGGGGCAGTGCATCTTTTGAAATCTAAAACGACTCTCGGCAACGGGTATTGGCTCT CGCATCGATAAAACGTAGCGAAATGCGATACTTTGGGGTGAATTGCCGATCTCCGTGAACCATCGAG TTTTTGAACGCTAGTAGGGCCAAAGCCATTTGGGCTAA
4	GACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCA AGTTGCGCCCGAGGCCGCTAGCTAGAGCACACGTGTGGTGGGTGTCACGCAATTCAACCCCCCTCCG CGTCTCTGCAGAGAGCAGAGCGAGATTGGTCCGTGGTGCATAGCCGGCGCGGTCTGAAATCAAGTAC GGGCGTCGCGTCATGGTTGAAGACCCCTTGCGAGACGGGGCCTGCCCGTCGGTGGGCCGTAAACTTG TATCGCTAGGGGCTTCTCGGAGGCGGATTTAGCTCCATCCATCTCCAGGGAAAGTAAATAAATTAGC GAAAAAAAAAAAAAC
5	ACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAA GGCGCCCTAGGCCATACGGTCGAGGGCACGTCTGCCTGGGCGTCACGCACTGGTGCCTCTCCCCTCCG ACGCCCCGTCCGGCTTCTGGAGCGGGAGAGCGGATATTGGCCCCCCGCGTGCCCCCATGCGGTCGGC GTAAAAGCTGGCCGTCGGCGAAGCATCACGACAGTTGGTGGTTGACGAGTCCATTCTCCGAAGCCGG ATGTCGTGACCGTCTCTCGCGCCCCGACCCCCGGGCCTAAGGTCGCCCGGGGGTTTTAGGAAAAAAA AA

6	CNNNNNNNNNNTGNNTNNCGNATCTNTGANACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGA ATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAGGCTATCCGGCCGAGGGCACGCCTG CCTGGGCGTCACGCCTCGCGTCGCTCCGCGCACCCTGCCCCCCGTCCCGGGGAGGCGGCGGGCGCAG ATGCGGAGATTGGCCCCCCGTGCCTCACGGCGCGGCGGGCCGAAGTGCGTGCCGCCGGCCGGGACGG ACNCGGCGAGTGGTGNACGGACACGTGCGGCGNTCAACGTCGCCTCCGCCCCCCGGNNNNGGNGGT GNNTGCAAGGAACCCACCCCGAGCGCCCNTNGNAACACNNCCCNNNGNGGGGGGGGGCCCCCNNTT NNNNTTNNNTNTNTNNNTGGGNGNANAAAAAAAAANAA
7	NNNNNNNNNNNNNNGNNTCTNGNATCNNTGNNACGTAGCGAAATGCGATACTTGGTGTGAATTGCA GAATCCCGCGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGTTGAGGGCACGTC TGCCTGGGCGTCTCACGTTGCGTCGCCCGCGCCCCCTCCCCCCCCTTTNTTTGGGGGAAGGANGGGCGG GGGGGGAAGTTGGNCCCCCGCCCCCCCCCCGCCCNCCCCAAAAAGAANCCCCNGGNNNGGGNNNNN NGGNGGGAAAAGGGGGGGNTGAAAAANCNTTNCNNNNCCNNNNNCNNNTNCNGGCCGTNTTGGGA AGNCNNTTNNGAACCTTTTNNGCCCCCCCNAANNNAANNNNNNCNNNNNNNNTCTNTTNNNNGGGG GGGGGNGNNNNTNNTTTTTTNNNNNNNNNNNNNNNNNNANNNNNNNNNNNNNN
8	NNNNNNNNNNNNANNNNNNNNNGNANNNNNNNNNGAACGTAGCGAAATGCGATACTTGGTGTGAA TTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCAAAGCCATTAGGCTAAGGGC ACGTCTGCCTGGGCGTCACGCATCCCGTCGCCACCAACCCCAATGCCCAGTCGGATATTGCGGGAGTT GGGGGCGGAAATTGGCCTCCCGTTCACGAACGTGCGCGGTTGGCCCAAAAAATGAGTTCTTGACGAT GGACGTCACGACAAGTGGTGGTTGAAAGACCTCTTGCGTCATGTCGTGAGGCACCAAGTCTGTAGCG AGCTCTGACCGCGACCCTGTGCACCCTTCTTCACGGATGGTGCTCCGACCGCGACCCCNGGTCAGACG GGATTACCCGCTGAGTTTAAGCATATCAATAAGCGGAGGAAAAAGAANNTNN
9	NNNNNNNNNNGGCTCNCGCATCTATGANNNGTAGCAACTGCGATACTTGGTGTGAATTGCAGAATCC CGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCACTAGGCCGAGGGCACGTCTGCCTG GGCGTCACGCATCGCGTCGCCCCCCCCCACGCCTCGCCCCGAACGGGACGAGGGTCGTGCGTGAGGGC GGAAAATGGCCTCCCGTGCTCCGTTGCGGCCGGCCTAAACCCGAGTCCCTCGCTGCGGACGTCACGA CGAGTGGTGGTTGAAACACTCAACTCGAATGCGAGTCGTGCGCGCCCNTGGCTGGGGATACCGTTAG ACCCTATGGCGAGCCCCTCNCGAGGGGNGCTCGCCACGACCGCGACCCCTGGTGAGGCGGGGNTACC CGCTGAGTTTTTGCNTATCANTGAGCGGAGNAAAAAAAANNANA
10	NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGACGTAGCGAAATGNGATACTTGGTGTGAATTGC AGAATCCCGCGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGTTGAGGGCACGT CTGCCTGGGCGTCTCACGTTGCGTCGCCCGCGCCCCCTCCCCCCCCTCCCCCAAAACGGGTGGAAANGA GGGCCGCGGGCGGATGTTGGCCCCCCNCGCGCNCNCCCGGNCCAAAATCGAGTCGGCNGCGACNGA CGACGCGTCGNNNNAGTGGAGNTTGACAAANCGTTNCGTCGCCTCGCNCCCGNCNGGNNGTCTCGAA NNNTNNGNNTTCTANNNCTCGCNNNGGCATNNGTCTNATNNNNGTNNNGGGGNGGNCNNTGANTTN NNTNTATNAGNNAAANAAANNAANNNN
11	NNNNNNNNNNNNNNNNGNNNNNNNNNNNNNNNNNNNACGTAGCANACTGTGATACTTGGTGTGAATT GCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGCCAAGGGCAC GTCTGCCTGGGCGTCACGCATCGCGTCGCCCCCCCACCCCAGTGGTGTCGAGGCGGAAAATGGCATC CCGTGGTCTGTCGCGGCCTGCCTAANNNNGTGTCCCTCGTCGCGGACGTCACGACAAGTGGTGGTTG AAATCCTCAACTCGAATGCNAGTCGTGCGCACCTCGTGGCCNAGACGACACNTAATAGACCCTTAGA CGATTCCCTTTCGAGGGAGGAGCATACGTCATGACTGCGACCCCNGGTCNGGNGGGGCTACCNNNTG ATTTTAAGCATATCAATAAGCGGAAANAAAAAAAAATA
12	NNNNNNNNNNNCGNCTCTCGCATCNATGNNGANCGTAGCGAAATGCGATACTTGGTGTGAATTGCAG AATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCAAAGCCATTAGGCTAAGGGCACGTCT GCCTGGGCGTCACGCATCCCGTCGCCACCAACCCCAATGCCCAGTCGGATATTGCGGGAGTTGGGGG CGGAAATTGGCCTCCCGTTCACGAACGTGCGCGGTTGGCCCAAAAAATGAGTTCTTGACGATGGACG TCACGACAAGTGGTGGTTGAAAGACCTCTTGCGTCATGTCGTGAGGCACCAAGTCTGTAGCGAGCTCT GACCGCGACCCTGTGCACCCTTCTTCACGGATGGTGCTCCGACCGCGACCCCNGGTCAGACGGGATT ACCCGCTGAGTTTAAGCATATCANTAAGCGGAGGAAAAAAAATAN
13	NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNACNNNCGTAGCGAAATGCGATACTTGGTGTGAATT GCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCTAGGCCATACGGTCGAGGGCAC GTCTGCCTGGGCGTCACGCACTGCGTGCCTCTCCCCTCCGACGCCCCGTCCCGGGCTTCTGGAGCGGG AGAGCGGATATTGGCCCCCCGCGTGCCCCCGGCATGCGGTCGGCGTAAAAGCTGGCCGTCGGCGGCG AAGCATCACGACAGTTGGTGGTTGACGAGTCCATTCTCCGAAGCCGGATGTCGTGACGGCGGCGTCG TCCTCGCGGCCCCGCGACCCCCCNNNNGGCCNNNNTNNNAAAGGGNTNCNNNCNGNNACCCCCGGT NNGGGGGGNNNNCNNNTTNNTTTTANCNTTTNATNAGNGGNNNAAAAAAAAANNN
14	NNNNNNNNNCGGCTCTCGCATCTATGANGNNGTAGCGAAATGNGATACTTGGTGTGAATTGCAGAAT CCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCAAAGCCATTAGGCTAAGGGCACGTCTGCCT GGGCGTCACGCATCCCGTCGCCACCAACCCCAATGCCCAGCCGGATATTGCGGGAGTTGGGGGCGGA AATTGGCCTCCCGTTCACGAACGTGCGCGGTTGGCCCAAAAAATGAGTTCTTGACGATGGACGTCAC GACAAGTGGTGGTTGAAAGACCTCTTGCGTCATGTCGTGAGGCACCAAGTCTGTAGCGAGCTCTGAC CGCGACCCTGTGCACCCTTNTTCACGGATGGTGCTCCGACCGCGACCCCNGGTCAGACGGGATTACCC GCTGAGTTTAAGCNTATCNNTAAGCGGAGNAAAAAAAAAANNAAN

15	NNNNNNNNNNNNNNNNNNNANNNNNNNNNACGTAGCGAAATGCGATACTTGGTGTGAATTGCAG AATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCAAAGCCATTAGGCTAAGGGCACGTCT GCCTGGGCGTCACGCATCACGTCGCCCCCAACCCCAAATGCCTTGATGTTGCGGGAGTTGGGGGCGG AAATTGGCCTCCCGTCCACACGACCGTGAGCGGTTGGCCCAAAAAATGAGTTCCTGACGAAGGACGT CACGACAAGTGGTGGTTGAAAGACCTCTTGCATCATGTCGTGAGGCGCCTAGTCTGTAGCGAGCTCTA ACCGTGGACCCTGCGCACCTAATTCGTTCCCGAAGGANANCGACGACGGTGCTTCGACCGCGACCCC CGGTCAGACGGGATTACCCGCTGAGTTTNAGCATATCAATAAGCGGAGGAAAAAAAAANNAN
16	NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNTTNNNCNNCCANCCCNNNNNNNNNGN NTTTNNNNNNTNTNNANNCCTTTTTTGAAAGAAAANANCGNNNGNNATAGTGAAATTTTCAGNNGTG CNNNTNNNTTACNAAAANGGNNCGNCTANNNANNNGCCNGCGGNNNNNNNAAAAN
17	NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCGTAGCGNNNATGCGATACTTGGTGTGA ATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCAAAGCCATTAGGCTAAGGG CACGTCTGCCTGGGCGTCACGCATCACGTCGCCCCCAACCCCAAATGCCTTGATGTTGCGGGAGTTGG GGGCGGAAATTGGCCTCCGTCCACACGACCGTGAGCGGTTGGCCCAAAAAATGAGTTCCTGACGAAG GACGTCACGACAAGTGGTGGTTGAAAGACCTCTTGCATCATGTCGTGAGGCGCCTAGTCTGTAGCGA GCTCTAACCGTGGACCCTGCGCACCTAATTCGTTCCCGAAGGAGAGCGACGACGGTGCTTCGACCGC GACCCCNGGTCAGACGGGATTACCCGCTGAGTTTAAGCATATCAATAAGCGGAAGAAAAAAAANN
18	NNNNNNNNNNNNGANNNNNGNANNNNNNNNAGACGTAGCGAAATGCGATACTTGGTGTGAATTGC AGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAGGCTATCCGGCCGAGGGCACGC CTGCCTGGGCGTCACGCCTCGCGTCGCTCCGCGCACCCNGCCCCCCTTCCCGGGGAGGCGGCGGGCG CAGATGCGGAGATTGGCCCCCCGTGCCTGACGGCGCGGCGGGTCGAAGTGCGTGCCGCCGGCCGGGA CGGACGCGGCGAGTGGTGTACGGACACGTGCGGCGCTCAACGTCGCCTCCGCCCCCNGTCCCGGNGN NNCATGNNNGNAACCCACGCCCNAGNNCCCCTTGGAAAANGANCCCNNGNNGGCGGGGCCACCCNN NGAGCTTNANNNTNTNGATTANNCGANGAAAAANAAACNAN
19	NNNNNNNNNNNNGNNNNNNGCANCNNNNNNGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCA GAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCAAAGCCATTAGGCTAAGGGCACGTC TGCCTGGGCGTCACGCATCCCGTCGCCACCAACCCCAATGCCCAGTCGGATATTGCGGGAGTTGGGG GCGGAAATTGGCCTCCCGTTCACGAACGTGCGCGGTTGGCCCAAAAAATGAGTTCTTGACGATGGAC GTCACGACAAGTGGTGGTTGAAAGACCTCTTGCGTCATGTCGTGAGGCACCAAGTCTGTAGCGAGCT CTGACCGCGACCCTGTGCACCCTTCTTCACGGATGGTGCTCCGACCGCGACCCCNGGTCAGACGGGAT TACCCGCTGAGTTTAAGCATATCAATAAGCGGAGGAAAAAAANNNANA
20	NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGTAGCAACTGTGATACTTGGTGTGAATTGC AGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGCCAAGGGCACGT CTGCCTGGGCGTCACGCATCGCGTCGCCCCCCCACCCCAGTGGTGTCGGGGCGGAAAATGGCATCCC GTGGTCTGTCGCGGCCTGCCTAAACCCGTGTCCCTCGTCGCGGACGTCACGACAAGTGGTGGTTGAAA TCCTCAACTCGAATGCGAGTCGTGCGCACCTCGTGGCCGAGACGACACGTAATAGACCCTTAGACGA TTCCCTTTCGAGGGAGGAGCATACGTCATGACTGCGACCCCAGGTCAGGCGGGGCTACCCGCTGAGT TTAAGCATATCAATAAGCGGAGGAAAAANAAANNN
50	NNNNNNNNNNNNNNNNNNNANNNNNNNNNNNNAGACGTAGCGAAATGCNGATACTTGGTGTGAAT TGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCAAAGCCATTAGGCTNNAGGGC ACGTCTGCCTGGGCGTCACGCATCCCGTCGCCACCAACCCCAATGCCCAGTCGGATATTGCGGGAGTT GGGGGCGGAAATTGGCCTCCCGTTCACGACCGTGCGCGGTTGGCCCAAAAAATGAGTTCTTGACGAT GGACGTCACGACAAGTGGTGGTTGAAAGACCTCTTGCGTCATGTCGTGAGGCACCAAGTCTGTAGCG AGCTCTGACCGCGACCCTNTNCACCCTTCTTCACGGATGGTGCTCCGACCGCGACCNCNGGTCAGACG GGATTACNNGCTGAGTTTAAGCGTATNANTAAAACAAAAANAAAAAGN

APPENDIX B

SAMPLE SUSPECT FINGERPRINT SHEET



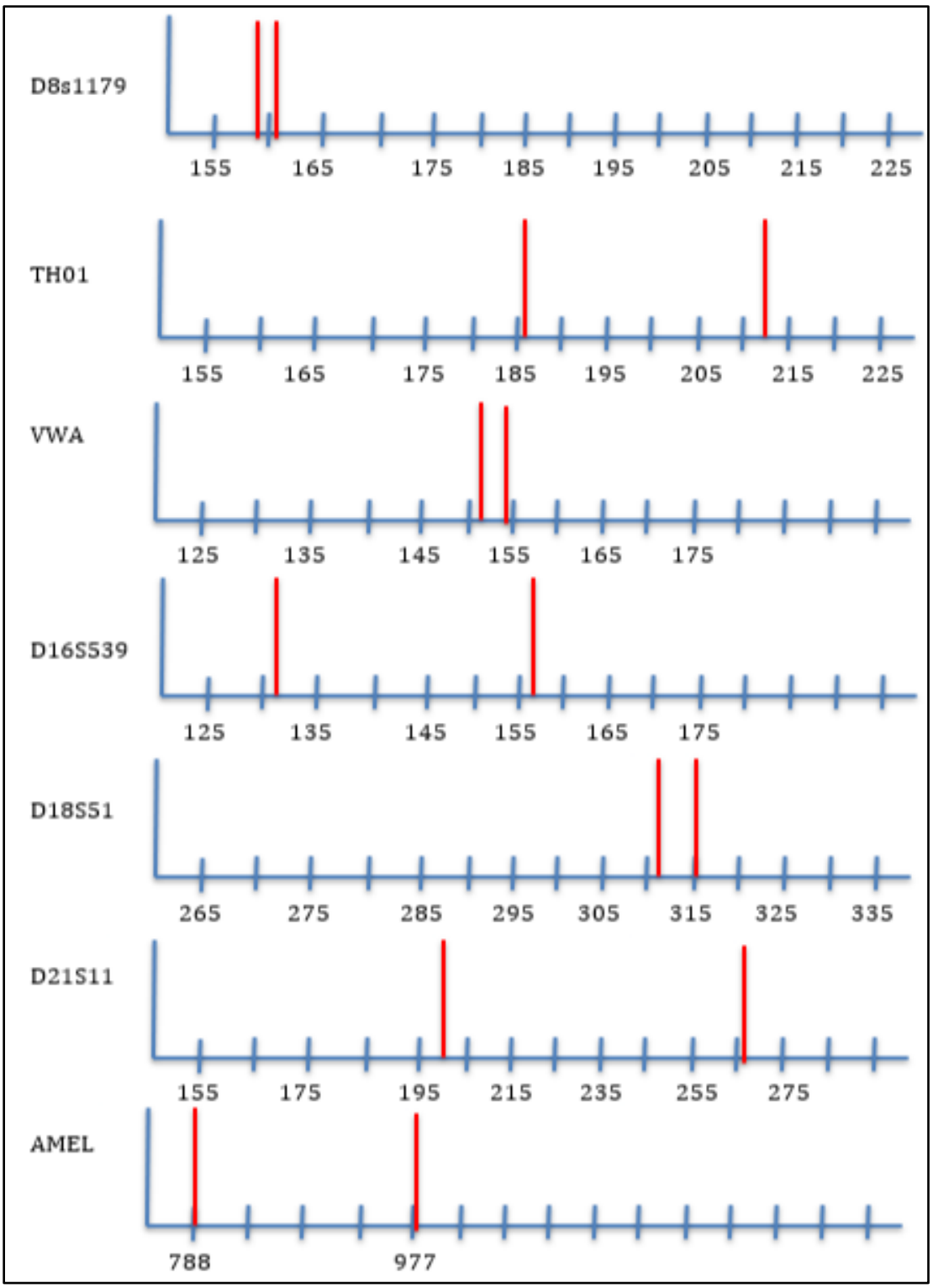
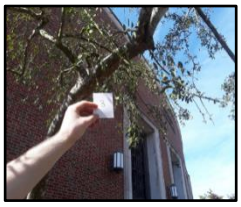


Figure A1. Sample suspect fingerprint sheet.

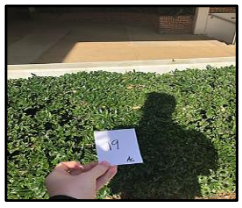
APPENDIX C

COLLECTION PICTURES & LOCATIONS OF PLANT SAMPLES

Table A2. Collection Pictures and Locations of Plant Samples

Plant Number:	Student Plant Picture:	Google Plant Picture:	Approximate Location
0			Quadrant IV; On border of Quadrant IV and Quadrant I
1	BLAST Failed	BLAST Failed	BLAST Failed
2	BLAST Failed	BLAST Failed	BLAST Failed
3			Quadrant I; In front of Howard
4			Quadrant I; In front of Howard
5			Quadrant I; On border of Quadrant I and Quadrant IV

6			Quadrant II; In front of University Hall
7			Quadrant II; In front of University Hall
8			Quadrant II; In front of University Hall
9			Quadrant II; In front of University Hall
10			Quadrant II; In front of University Hall
11			Quadrant III; In front of Wiley Tower
12			Quadrant III; In front of Wiley Tower

13			Quadrant III; Between Wiley and Library
14			Quadrant III; In front of Library
15			Quadrant III; By Library, on border of Quadrant II and Quadrant III
16	PCR Failed	PCR Failed	PCR Failed
17			Quadrant IV; In front of Howard
18			Quadrant IV; In front of Howard, next to Book Store
19			Quadrant IV; In front of Book Store

20			Quadrant IV; Around bottom of tree in front of Book Store
50			Quadrant II; In front of Library, between Library and diagonal sidewalk

APPENDIX D

COMPLETE LIST OF MATERIALS

Table A3. Complete List of Materials

Item	Supplier	Catalog Number
Plasticware and Disposables		
Eppendorf Tube racks 96 place, multiple colors available	VWR	82024-488;486; 490; 492; etc
Eppendorf tubes, 1.5 ml flip top	VWR	89000-028
Eppendorf tubes, 2.0 ml flip top - asst	VWR	20170-098
Latex gloves – extra large	VWR	82026-422
Latex gloves – large	VWR	82026-420
Latex gloves – medium	VWR	82026-418
Latex gloves – small	VWR	82026-416
PCR plate holder with lid, 96 well assorted colors	VWR	80086-074; 076; 078; 080; etc
PCR plate: 96 well	VWR	47744-106
PCR tubes: 12 with separate caps	VWR	53509-306
Pipette rack, 3 place acrylic	VWR	82024-540
Pipette tips 1000 uL blue	VWR	83007-376
Pipette tips 200 uL yellow	VWR	53508-810
Screw cap tubes, 1.5 ml	VWR	89004-292
Chemicals and Reagents and Media		
Agarose	VWR	JTA426-7
Biorad Instagene matrix	Biorad	
Boric Acid (55 g/L of 10X TBE running buffer)	VWR	EM-2710
DNTPs set of 4: 100 mM each	VWR	PAU1330
EDTA (9.3 g/L of 10X TBE running buffer)	Fisher	BP-120
Ethidium Bromide	Fisher	BP1302-10
GoTaq green polymerase (Promega)	VWR	PAM7122
Lambda DNA (Promega)	VWR	WLBPAZD1501
Mineral oil (In the health and beauty section of most chain stores)	Local	
Molecular Biology grade water	VWR	12001-380

TE ($Tris_{10}$:$EDTA_1$) (Promega)	VWR	PAV6231
Tris Base (104 g/L of 10X TBE running buffer)	Fisher	BP-154
LB agar plates	VWR	100216-614
TSA agar plates	VWR	101320-676

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